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Pharmacological and Toxicological Studies on Salicylamide*

By C. T. ICHNIOWSKI and W. C. HUEPER

The acute and chronic toxicities, pharmacologic and hematologic effects of salicylamide have been studied. Aspirin served as a comparative control. Salicylamide exerts a moderately quicker and deeper depressing effect than aspirin. Oral daily feeding of one-fourth the single lethal dose for a period of thirteen weeks did not induce any untoward symptomatic and anatomic reactions. Data presented suggest that salicylamide appears to differ in its metabolization from other salicylic compounds. The oral lethal dose of salicylamide in rats equals that of aspirin.

SALICYLAMIDE is a salicylic acid derivative known for approximately fifty years but apparently rarely used as a medicinal agent. While salicylic acid and its derivative, aspirin, are well known for their antipyretic, antirheumatic, and analgesic action, the scanty experimental data available on the pharmacologic effects of salicylamide emphasize its narcotic activity.

Meyer (1) stated that, on tadpoles, salicylamide is more efficient as a narcotic at 3° than at 30°. At both these temperatures salicylamide has a higher "narcotic rating" because of its respective partition coefficients (oil-water) than ethyl alcohol, chloral hydrate, acetone, and benzoic amide. Collett (2) using salicylamide on lower zoological forms (*Loligo, Gonionemus, Perphora*) found that the lipoid theory of narcosis held at weak dilutions, but was complicated by other factors when stronger solutions were employed. Kitano (3) reported progressive narcotic activity with increasing temperatures (up to 39°), when a 1:500 salicylamide solution was tested on the isolated extensor communis muscle of rabbits. Anselmino (4), when investigating the changes in the electromotive activity of collodion membranes by narcotics, observed that variations in potential resulted from the use of salicylamide and compounds of the urethane series.

Because of the possibility that salicylamide might combine the antipyretic and analgesic properties of the ordinary salicylic compounds with narcotic ones, it was decided to study its effects upon warm-blooded

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animals. Aspirin was used as a comparative control for the experiments.

EXPERIMENTAL

Salicylamide or *o*-hydroxybenzamide is a practically tasteless, yellowish white, crystalline powder that is somewhat more soluble in water than salicylic acid, and which has a melting point at 138°. As it contains an amide group replacing a —OH group in the carboxylic chain, it should be less irritating than either free salicylic acid or sodium salicylate, as the amide group is not likely to split off as readily in the gastric secretion as sodium from sodium salicylate.

Since salicylamide is relatively insoluble in highly aqueous media, several experiments were carried out in order to devise a solvent capable of dissolving salicylamide to a much greater degree than water. Negative results were obtained with a 20% aqueous solution of propylene glycol and a 20% aqueous solution of propylene glycol plus a 20% aqueous solution of ethanol. In a menstruum containing 4 parts of propylene glycol and 1 part of ethanol, as much as a 10% solution of salicylamide could be obtained. However, preliminary experiments with this menstruum indicated that the doses of the menstruum necessary for the administration of adequate amounts of salicylamide would complicate the analysis and interpretation of the narcotic and analgesic action possibly exerted by the drug and in themselves would be irritating because of the hygroscopic nature of these solvents.

Salicylamide solutions prepared by making alkali combinations of the amide in a watery medium using sodium carbonate or triethanolamine left much to be desired, since these solutions turned yellow or yellow-red within an hour and formed a precipitate at times. These changes indicated that decomposition had occurred, making the solutions unsuitable for experimental purposes.

The preparation finally adopted consisted of a suspension of salicylamide in a 0.5% solution of gum tragacanth, which was administered orally and intraperitoneally to rats. Aspirin was given in the same manner.

Table I.—Toxicity of Salicylamide After Oral Administration

Rat Group	Dose, Mg./Kg.	24-Hr. Toxicity	4-Day Toxicity	Comments[a]
1	400	0/5	0/5	Throughout the day of the administration all were responsive and active. On the days following administration the animals behaved normally
2	600	0/5	0/5	Approximately one hour after administration the group was depressed and showed hind leg incoordination. Recovery was noted three hours after administration. On successive days the animals behaved normally
3	800	0/5	0/5	Two of the animals were heavily depressed and gave no response to tail pinches. The other three acted normally. Five hours after administration the whole group was normal and behaved normally on the succeeding days
4	1000	0/5	0/5	Similar to Group 3, but depression lasted until end of work day. Recovery was complete on the following morning. Thereafter the animals were normal in activity and response
5	1600	0/5	0/5	Four of the rats showed heavy depression and the remaining one a mild depression approximately one hour post-administration. Response to tail pinch was negative. Recovery ensued following morning. Thereafter the rats acted normally
6	2000	1/5	1/5	One rat died within two hours post-administration. The remaining 4 were heavily depressed and yielded no response to tail pinch. The effect lasted during work day. Recovery noted by next morning and animals behaved normally thereafter

[a] The depression did not appear to be a narcosis-like symptom.

Table II.—Toxicity of Aspirin after Oral Administration

Group No.	No. of Rats in Group	Dose, Mg./Kg.	24-Hr. Toxicity	4-Day Toxicity	Comments
1	5	400	0/5	0/5	All the surviving animals were in good condition barring a temporary distress in the heavier dosed groups, shortly after administration. Recovery was rapid; within thirty minutes. The animals thereafter and until end of observation period of four days acted normally
2	5	600	0/5	0/5	
3	5	800	1/5	1/5	
4	15	1000	1/15	2/15	
5	10	1600	1/10	1/10	
6	10	2000	0/10	0/10	

A. Acute Toxicity: Oral Administration.—In a preliminary experiment 12 groups of rats, containing 5 animals each, were given doses of 0.4 to 2.0 Gm./Kg. of the two drugs. The salicylamide as

well as the aspirin was administered as a 20% suspension in tragacanth solution by means of a hypodermic syringe with a blunted needle. Six groups were employed for the testing of each drug. The results obtained are tabulated in Tables I and II.

After the approximate lethal dose of salicylamide had been thus ascertained, 3 groups of 15 rats each were tested with dose levels of 0.5, 1.0, and 2.0 Gm./Kg. of salicylamide and aspirin, respectively, given as a 20% suspension in 0.5% tragacanth solution. The results obtained are presented in Tables III and IV.

The autopsies of the rats which were treated with either drug and which died during the period of observation exhibited congestion and hemorrhages of the lungs and hyperemia of the meninges. All other organs were grossly normal.

B. Chronic Toxicity: Oral Administration.—Five groups of 20 rats each (10 males and 10 females) weighing between 100 and 130 Gm. were placed on chronic salicylamide and aspirin medication, respectively. The dose levels of the two drugs given were 5, 25, 50, 100, and 200 mg./Kg., five times per week, for thirteen weeks, with a total of 67 doses.

TABLE III.—LETHAL ORAL DOSE OF SALICYLAMIDE IN RATS

Group No.	Rat No.	Dose, Mg./Kg.	24-Hr. Toxicity	1-Wk. Toxicity	Comments
1	1–15	500	0/15	0/15	Rats showed no evidence of narcosis or depression; they responded normally for several hours after administration and reacted normally for remainder of observation period
2	16–30	1000	0/15	0/15	Rats behaved and reacted like those in Group 1
3	31–45	2000	9/15	9/15	Seven died within one hour after administration; seven went into a heavy depression, while the remaining ones of the group reacted and responded normally. Two more rats died overnight after the administration, giving a 60% mortality in twenty-four hours

TABLE IV.—LETHAL ORAL DOSE OF ASPIRIN IN RATS

Group No.	Rat No.	Dose, Mg./Kg.	24-Hr. Toxicity	1-Wk. Toxicity	Comments
1	40–60	500	0/15	2/15	Animals were in good condition after administration and showed no symptoms of depression. For the period of observation the animals reacted normally
2	61–75	1000	5/15	9/15	No depression for several hours. Deaths overnight
3	76–90	2000	8/15	12/15	No depression for several hours. Deaths overnight

The autopsies performed on the rats of both groups which died during the first week after the administration of the drugs revealed in all animals congestion and edema of the lungs, which findings in some cases were associated with local hemorrhages, and hyperemia of the liver. All other organs were grossly normal. The approximate *LD* for both drugs is thus in the neighborhood of 2 Gm./Kg. Salicylamide appears to cause a distinct depression more quickly than aspirin, which in turn seems to be somewhat more toxic than salicylamide.

Intraperitoneal Administration.—Since the depression noted in the oral salicylamide groups did not appear like a narcosis, it was decided to repeat the administration of salicylamide and aspirin by the intraperitoneal route. A 10% suspension of these drugs in a 0.5% solution of gum tragacanth was injected into four groups of rats, of 10 animals each, at four dose levels ranging from 0.25 to 1.5 Gm./Kg. Tables V and VI show the results of this study.

It is obvious from these data that aspirin intraperitoneally given is more toxic than salicylamide, despite the fact that the latter drug elicits a more rapid and a deeper depression than the former. Throughout the observation period there was no evidence of a narcotic action of salicylamide, even when given in doses of 1000 mg./Kg.

The amounts administered correspond to approximately 1, 5, 10, 20, and 40 times the average human adult dose. The total quantity of salicylamide given to an individual rat ranged between 70 mg. and 13,400 mg., while that of aspirin varied from 75 mg. to 13,800 mg. The volume administered per gram of rat was either 0.01 cc. or 0.02 cc. The medicaments were suspended with the aid of a 0.5 per cent tragacanth solution and given with a syringe.

All animals dying during the course of the experiments or sacrificed afterward were subjected to a post-mortem examination. Some of the surviving rats were sacrificed at the end of the experimental period for hematologic studies, while others were sacrificed by the intravenous injection of a 4% formaldehyde solution three to seven weeks after the cessation of medication. A histologic examination of the internal organs was made of 46 rats which received salicylamide and of 44 rats given aspirin. The hematologic examination consisted of the determination of the prothrombin time, coagulation time, amount of hemoglobin, counts of the leucocytes and erythrocytes, and of differential counts whenever an abnormal number of leucocytes was found. The blood for these tests was obtained from the jugular vein. The weight of the rats was determined weekly for eighteen to twenty weeks.

Deaths occurred during the course of the experi-

ment to about an equal degree in all groups and thus at all dose levels with both drugs. Inasmuch as in all these cases the autopsy showed hyperemic and hemorrhagic lungs and a seropurulent pleurisy and pericarditis, it is likely that these fatalities are not attributable to any toxic action of the chemicals given, but to technical accidents connected with their administration, either represented by a direct injection of the suspension into the lungs or by a secondary regurgitation of the injected matter into these organs. This conclusion is supported by the fact that all rats treated with either drug and killed at the end of the experimental period did not exhibit any significant abnormal changes upon gross and microscopical examination of their internal organs. Neither salicylamide nor aspirin in the doses given thus seemed to have exerted any anatomically demonstrable toxic toxic or lethal effect.

time of the cessation of treatment with salicylamide ranged from 200 to 310 Gm., and fluctuated between 240 and 370 Gm., four weeks later, while the corresponding figures for the female rats were 160 to 205 Gm., and 180 to 235 Gm., respectively. In the male aspirin rats the weights ranged from 250 to 320 Gm., at the cessation of treatment and from 270 to 350 Gm., four weeks later, whereas the corresponding figures for the female rats stood at 180 to 215 Gm., and at 185 to 240 Gm., respectively. It is uncertain whether these differences in the weight curves of the two sexes are merely sex conditioned or represent differences in reaction of the two sexes to a toxic action of the two salicylic compounds.

The hematologic studies showed that the prothrombin times of the 19 rats of the salicylamide series were within normal limits (average from 29.26

TABLE V.—INTRAPERITONEAL TOXICITY OF SALICYLAMIDE

Group No.	Rat No.	Dose, Mg./Kg.	24-Hr. Toxicity	1-Wk. Toxicity	Comments
1	1–10	250	0/10	1/10	All rats were heavily depressed fifteen minutes after administration. Within two hours all 10 rats had recovered. The animals continued in good condition one week after administration
2	11–20	500	2/10	2/10	All were readily depressed in fifteen minutes after administration. Five hours after administration the 8 survivors had recovered and continued in good condition for one week. Two died within two hours after administration. During period of depression the rats exhibited a hind leg paralysis
3	21–30	1000	9/10	9/10	All in the group reacted very readily, going into a heavy depression and coma-like condition. Nine of the rats died; 7 within thirty minutes after administration and the other 2 died within one and one-half hours after administration. The one survivor recovered from the depression within five hours after receiving the drug. This survivor continued in good condition for the remainder of the observation period of one week
4	31–40	1500	10/10	..	All 10 rats went into a heavy depression almost immediately. Seven died ten minutes after administration and the other three within one hour later

TABLE VI.—INTRAPERITONEAL TOXICITY OF ASPIRIN

Group No.	Rat No.	Dose, Mg./Kg.	24-Hr. Toxicity	1-Wk. Toxicity	Comments
1	1–10	250	0/10	0/10	No acute symptoms discernible; all were in good condition and remained thus for one week
2	11–20	500	4/10	9/10	Slight depression set in within two hours after administration; complete recovery three hours later. Survivors after twenty-four hours were in good condition. Four deaths within twenty-four hours; 3 more died during third day; one additional rat died on fourth day, and the ninth rat died on seventh day
3	21–30	1000	10/10	10/10	All ten died within two hours after administration
4	31–40	1500	10/10	10/10	All ten died within three hours after administration

The weights of the rats of both series and of all dose levels increased steadily during the course of treatment to about an equal degree with the exception that the female rats gained weight less rapidly than the male and, particularly, did not show the very rapid elevation of weight after the discontinuation of medication, which was seen in the male rats of both series. The weights of the male rats at the

seconds to 36.55 seconds) after sixteen weeks of medication. The coagulation times were relatively high in many instances with the averages of the different groups fluctuating between five and seven minutes. The erythrocytic and hemoglobin values were at the lower border of the normal range. The number of leucotytes, on the other hand, ranged in three animals between 300 and 5300 and was above

10,000 in only 2 other animals. The differential counts did not reveal any consistent differences between the rats with a normal number of leucocytes and those with a leukopenic reaction.

The prothrombin times of the aspirin rats ranged from averages of 15.8 seconds to those of 33.1 seconds without showing any relation to the dose levels. The coagulation times fluctuated between averages of four minutes and two seconds, and six minutes and thirty-one seconds. The erythrocyte and hemoglobin values were well within the normal range and in some rats were even slightly above the normal limit. The number of leucocytes varied between 7000 and 17,600 and was in most rats above 10,000. The differential counts exhibited normal variations.

In view of the leukopenic reactions observed in a few rats of the salicylamide series, a second chronic experiment was initiated under carefully controlled conditions for the purpose of studying more thoroughly the hematic reactions of rats exposed to salicylamide over long periods of time. Again both salicylamide and aspirin groups were used. The individual groups contained 10 rats (5 males, 5 females). Three dose levels, 5, 50, and 200 mg./Kg., given five times weekly, were used.

The medicaments, as in the previous experiment, were given in 0.5% tragacanth suspension. Blood samples were obtained from the jugular veins of the animals which were kept under light ether anesthesia during the sampling process. A sampling schedule was so arranged that the blood specimens were obtained bi-weekly from both the aspirin and salicylamide rats. The females and males were so alternated that the bi-weekly specimens of blood were obtained and any given rat was not used for sampling but once every four weeks. A 1-cc. sample of blood was obtained from each rat for purposes of the routine hematologic studies which included red and white cell counts, hemoglobin determinations, differential counts, and prothrombin time. A control blood specimen was obtained for analysis from each rat prior to medication. The medication course lasted for thirteen weeks, during which period the rats surviving had received 67 doses. The total amounts received by the survivors of the three groups on each medication of 5 mg., 50 mg., and 200 mg./Kg., per day were 335 mg., 3350 mg., and 13,400 mg., per Kg., respectively. Representative numbers of each group were sacrified after the medication course for gross pathologic examination.

An appreciable number of rats died during the course of the experiment as the result of technical accidents. Several animals in the aspirin series receiving 200 mg./Kg. developed "cauliflower ears" during the latter part of the experiment which were transformed into large blisters of pink to bluish red color and up to a small navy bean size. In several instances these blisters receded after some time and the affected ears became distorted by cicatrization.

The hematological data of both series did not reveal any significant changes from the normal range with the exception that again the rats of the salicylamide group had low normal values as to hemoglobin and erythrocytes, while the animals in the aspirin group revealed higher figures in these respects. There were no leukopenic reactions among the animals treated with salicylamide. The prothrombin times fluctuated considerably during the course of the experiment among the rats of both series, but there was no progressive or consistent trend toward a hypoprothrombinemia.

The post-mortem examination of the rats which died during the course of the experiment showed congested and hemorrhagic lungs and meninges and, often, seropurulent fluid in the thoracic cavities. The histological examination of the organs of the rats sacrificed at the end of the experiment revealed no abnormalities. The "cauliflower ears" exhibited a large central cavity filled with plasma and erythrocytes and lined by endothelial cells. The overlying epidermis was hyperplastic, while the cartilaginous plate which formed a part of the cystic wall in some instances showed signs of liquefaction necrosis. The surrounding tissue contained distended veins and capillaries and hemorrhages. Regressed lesions consisted of an inflammatory granulation tissue with necroses.

C. Hypoprothrombinemia Study.—In view of the fact that claims have been made that a chronic and excessive use of aspirin or other salicylates may lead to the development of a hypoprothrombinemia, it became pertinent to determine whether or not salicylamide exhibits this influence and to the same degree as aspirin. Two groups of 25 rats each were therefore placed on a vitamin K deficient diet (casein 18 parts, yeast 8 parts, salts 4 parts, Wesson oil 5 parts, cod-liver oil 2 parts, cerelose 63 parts) for a period of ten days. The rats of one group received then, in about 5 Gm. of a mixture of cooked cornstarch containing 2% cotton seed oil, 100 mg. of aspirin daily. The second group of 25 rats was fed, in the same mixture, 100 mg. of salicylamide. The prothrombin time was determined before the start of these dietary managements and after six, twelve, and eighteen days thereafter. The blood was withdrawn from the jugular vein.

The average prothombin time of the salicylamide rats was 26 seconds before the start of the treatment and stood at 59 seconds at the end of the experimental period. The corresponding figures for the aspirin rats were 25.3 seconds and 53.4 seconds, respectively. There is thus no fundamental difference in the hypoprothrombinemic action of the two salicylic compounds.

D. Chemical Determination of Salicylamide.—Some preliminary work was carried out on the quantitative determination of salicylamide in blood for the purpose of being able to follow blood levels and the persistence of the drug in this medium. The colorimetric method of Brodie, Udenfried and Coburn (5) was applied involving the use of ethylene dichloride for the extraction of the "salicyl" preparation from the blood.

While the method worked well with sodium sal-

icylate and aspirin, doses up to 300 mg./Kg. of salicylamide given orally to dogs failed to give a positive reaction in the blood. The same negative result was obtained when other organic solvents (chloroform, benzol, carbon tetrachloride), possessing a different partition coefficient, were tried. Despite the fact that salicylamide contains a phenolic hydroxyl complex in its molecule like the other two salicylates, it differs in this respect from the ordinary salicylic acid compounds.

DISCUSSION

The various experiments showed that salicylamide has a stronger depressive action than aspirin, but does not elicit a state of narcosis as claimed on the basis of results obtained in *in vitro* experiments and in studies on isolated tissues. The oral lethal dose of salicylamide and aspirin for rats is in the neighborhood of 2000 mg./Kg., while the intraperitoneal lethal dose lies around 1000 mg./Kg. The lethal effect is associated with circulatory disturbances causing a congestion and hemorrhages of the lungs and meninges.

The prolonged, repeated oral administration of large doses of salicylamide and aspirin did not cause in rats any ulcerative lesions of the gastric mucosa, such as those reported by Barbour and Dickerson (6). Such reactions were seen regularly, on the other hand, in rabbits and dogs receiving daily by mouth 0.7 Gm./Kg. of aspirin for four to eighteen days, confirming thereby observations of Thompson and Dragstedt (7), and Barbour and Porter (8), made in dogs. Madisson (9) attributed this effect of salicylates to a phenol-like action of these chemicals upon mucous membranes.

The hematic studies indicate that salicylamide exerts approximately the same hypoprothrombinemic effect as aspirin when given to rats kept on a low vitamin K diet. No such action was observed when these animals received stock diet. The presently available data do not permit a definite decision of the question as to whether or not salicylamide sometimes may produce a leukopenic reaction. The probability of the actual occurrence of such reactions, however, appears to be remote, if salicylamide reacts in this respect like other salicylic compounds which rarely or never exert such a response [Hawkinson and Kerr (10)].

The absence of any other significant untoward effect upon the blood and internal organs when salicylamide is given orally to rats in large doses over long periods of time placed this chemical alongside aspirin, which was shown to be equally harmless under similar experimental conditions [Robinson, Ellis, and Warner (11)]. The outcome of the chemical tests performed on the blood of dogs which had ingested appreciable amounts of salicylamide indicate, on the other hand, that this drug differs fundamentally in its *in vivo* reactivity from that of other salicylates.

CONCLUSIONS

1. Salicylamide exerts a moderately quicker and deeper depressing effect than aspirin.

2. The oral lethal dose of salicylamide in rats equals that of aspirin (2000 mg./Kg.).

3. Rats given one-fourth of the single lethal dose (SLD_{50}) daily for thirteen weeks do not exhibit any untoward symptomatic and anatomic reactions.

4. Salicylamide seems to differ in its metabolization from other salicylic compounds.

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The Anesthetic Effects and Toxicities of Some New Barbituric Acid Derivatives Administered to Mice and Rats. I*

By WILLIAM H. HUNT,† RUSSEL J. FOSBINDER, and O. W. BARLOW

Extensive data are reported on the time of onset of hypnosis, duration of action, and the toxicity to mice and rats for approximately 150 new 5,5-disubstituted barbituric and thiobarbituric acids. In general, the data indicate that the duration of hypnotic effect of the new compounds decreased as the molecular weight increased, and the barbiturates containing a sulfur-interrupted carbon chain in one of the substituent groups were more active than those not containing sulfur.

A SERIES of approximately 150 new 5,5-disubstituted barbituric and thiobarbituric acids, containing a sulfur interrupted carbon chain in one of the substituent groups, were prepared in the laboratories of one of us for the purpose of pharmacological examination. Walter, Goodson, and Fosbinder (1), in a series of papers published elsewhere, have described the methods of preparation of the new compounds and the appropriate intermediates.

All of the compounds prepared by Walter, *et al.*, have been examined experimentally by administration of the sodium salts to one or more species of animal. The data presented in this paper are confined to observations on the rat and the mouse and include information relative to the onset of hypnosis, the duration of effect as determined by various criteria, and the acute toxicity following intravenous injection in the rat and intraperitoneal injection in the mouse. Several of the new derivatives have been administered orally to rats in lethal and sublethal doses and the tolerance of this species of animal to repeated doses was determined.

EXPERIMENTAL

Duration of Effect and Toxicity—Rat.—Solutions of the sodium salts of the barbituric and thiobarbituric acids listed in Tables I, II, III, and IV, respectively, were injected in graded doses into the saphenous vein of male, albino adult rats weighing between 100 and 225 Gm. The volume of the dose administered was kept approximately constant by adjusting the concentration between 1 and 5%. In every instance a total of 0.1 cc. of solution was injected in an interval of fifteen seconds. Adult male rats were used throughout the experiments as other investigators (2, 3) have shown that inconsistent results are obtained with immature animals and that female rats exhibit a more prolonged hypnosis as compared to males.

The onset of hypnosis and the duration of various stages of anesthesia were determined by observing the abolition and reappearance of reflexes as described below:

Righting Reflex—the period during which the animal failed to right itself when placed on its side.

Corneal Reflex—response to the application of the blunt end of a feather applied to the cornea.

Ear Reflex—response to the application of the tip of a feather to the inner surface of the ear.

Tail Reflex—response to immersion of the tail in water at 58–60° for five seconds.

Although the period of loss of righting reflex ended when the animals were able to right themselves, the total period of barbiturate effect was often much longer as indicated by continued depression. In the determination of the mean hypnotic dose (MHD_{50}) the abolition of the ear reflex rather than of the righting reflex was employed as a criterion because a more consistent response was observed. All animals given sublethal doses of the compounds were used only after a seven-day rest period to permit full recovery from the effects of medication. In this study Pentobarbital Sodium, Evipal Sodium, and Pentothal Sodium were used as reference compounds.

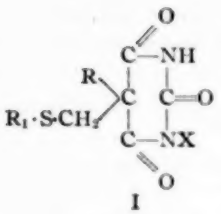
It will be seen from Tables I, II, III, and IV that when injected intravenously the new compounds induced characteristic hypnotic effects in common with the reference barbiturates. As is to be expected in an extensive series of derivatives, wherein freedom of substitution is substantial, wide variations in duration of effect, activity, and toxicity were obtained. Inasmuch as data on only one species of animal are being reported here, generalizations concerning the relationship of structure to pharmacologic action will be reserved for a later publication.

* Received Dec. 17, 1945, from the Research Laboratories of the Maltbie Chemical Co., Newark, N. J., and the Winthrop Chemical Co., Rensselaer, N. Y.

† Present address: Marvin R. Thompson Laboratories, Stamford, Conn.

Table V shows the results obtained with a selected group of compounds administered orally to rats in hypnotic and lethal doses. Male adult rats weighing between 175 and 300 Gm. were fasted for eighteen hours and graded doses of the sodium salts were then given by stomach tube. In the lower dosage range 5% solutions were employed, while the concentration was increased to 10% in the lethal range in order to restrict the volume. Five or 10 animals were used at each dosage level and the LD_{50} was estimated by the application of Kärber's method (4).

The formulas of the new compounds referred to by numbers in Tables I, II, III, IV and V are the following:



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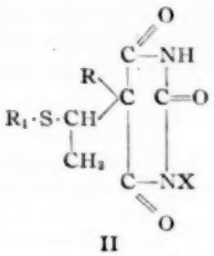

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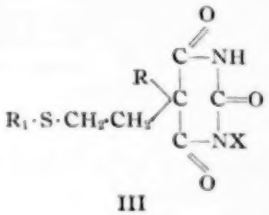

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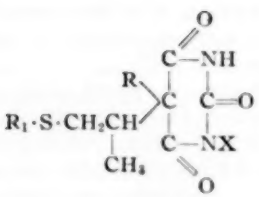

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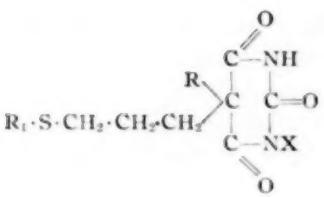
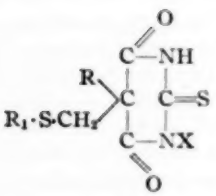


V



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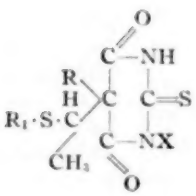

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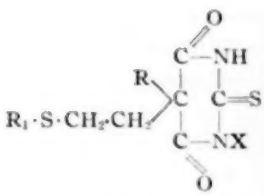

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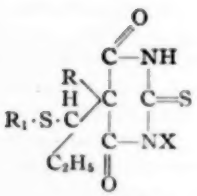

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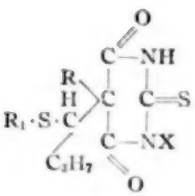

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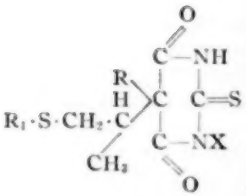


XI

TABLE I.—EFFECTS OF INTRAVENOUS INJECTIONS IN WHITE RATS OF VARIOUS SUBSTITUTED BARBITURIC ACID DERIVATIVES AS HYPNOTICS AND THE OBSERVED TOXICITY

Compound I		No. of Animals	Dose, Mg./Kg.	Down After Injection, Min.	Duration of Loss of Various Reflexes, Min.				
R	R_1				Righting	Corneal	Ear	Tail	Killed
Butyl	Ethyl	5	80	Immediate	67	+	8	+	0/5[a]
Allyl	Ethyl	6	40	Immediate	24	+	+	+	0/6
		6	60	0.7	72	+	22	+	0/6[b]
		6	80	1	93	32	28	18	0/6
		6	100	Immediate	125	39	104	31	0/6[b]
		6	120	0.9	133	20	62	37	2/6
Isoamyl	Ethyl	9	40	Immediate	8	+	+	+	0/9
		12	60	Immediate	12	+	+	+	0/12[c]
		12	80	Immediate	25	+	+	+	0/12[c]
		6	100	Immediate	30	+	2	+	0/6[a]
		12	120	Immediate	42	2	8	2	5/12
		6	140	Immediate	75	15	16	10	5/6
Isopropyl	Ethyl	6	60	Immediate	98	10	37	+	0/6
		6	80	Immediate	109	21	59	+	0/6
		6	100	Immediate	165	79	109	46	2/6
		6	120	Immediate	170	67	116	70	1/6
		6	140	Immediate	188	117	133	111	3/6
Isobutyl	Ethyl	6	20	Immediate	7	+	+	+	0/6
		6	40	Immediate	25	+	+	+	0/6
		6	60	Immediate	55	3	9	3	0/6
		6	80	Immediate	52	10	23	10	0/6
		6	100	Immediate	146	82	93	69	1/6
		6	120	Immediate	95	32	41	30	0/6
		6	140	Immediate	102	87	124	84	3/6
Secondary Butyl	Ethyl	6	20	Immediate	12	+	+	+	0/6
		6	30	0.5	37	+	+	+	0/6
		12	40	Immediate	30	+	+	+	0/6
		12	60	Immediate	50	+	9	+	0/6
		12	80	Immediate	82	26	41	37	1/6
		12	100	Immediate	140	68	75	83	1/6
		6	120	Immediate	263	133	175	173	2/6
		6	140	Immediate	276	105	175	194	1/6
		6	160	Immediate	272	175	175	159	2/6
1-Methylbutyl	Ethyl	6	20	1	7	+	+	+	0/6
		6	40	Immediate	25	+	3	+	0/6
		7	60	Immediate	52	9	29	+	0/7
		6	80	Immediate	67	29	40	13	0/6
		6	100	Immediate	99	51	70	36	0/6
		6	120	Immediate	121	57	74	67	3/6
		6	140	Immediate	139	71	85	69	5/6
Isobutyl	Methyl	6	60	Immediate	44	+	+	+	0/6
		6	80	Immediate	80	26	40	+	0/6
		12	100	Immediate	121	62	87	26	0/12
		12	120	Immediate	141	66	81	48	0/12
		12	140	Immediate	229	138	152	128	2/12
		18	160	Immediate	144	89	105	75	2/18
		6	170	Immediate	95	45	65	41	1/6
		12	180	Immediate	149	89	96	75	8/12
Secondary Butyl	Butyl	6	20	Immediate	13	1	4	+	0/6
		6	30	Immediate	16	+	7	+	0/6
		6	40	Immediate	23	5	14	4	0/6
		6	50	Immediate	29	7	19	11	0/6
		6	60	Immediate	35	14	17	14	0/6
		6	70	Immediate	51	12	24	16	0/6
		6	80	Immediate	...	...	...	...	6/6
Butyl	Butyl	6	20	Immediate	2	+	+	+	0/6
		12	40	Immediate	7	+	1	+	0/2
		6	60	Immediate	10	+	5	+	0/6
		6	80	Immediate	16	3	8	+	2/6
Ethyl	Amyl	6	40	Immediate	12	+	+	+	0/6
		6	60	Immediate	29	9	15	+	0/6
		6	80	Immediate	63	25	43	3	0/6
		6	100	Immediate	88	41	66	17	1/6
		6	120	Immediate	110	71	80	30	2/6
		6	140	Immediate	104	75	75	44	5/6
Isopropyl	Butyl	6	10	Immediate	4	+	+	+	0/6

[a] Constant muscle tremors [b] Slight tremor [c] Convulsive movement on injection

(*Cont'd on next page*)

TABLE I.—EFFECTS OF INTRAVENOUS INJECTIONS IN WHITE RATS, ETC., (*Cont'd from page 233*)

Compound I		No. of Animals	Dose Mg./Kg.	Down after Injection, Min.	Duration of Loss of Various Reflexes, Min.				
R	R_1				Righting	Corneal	Ear	Tail	Killed
Isopropyl	Butyl	6	20	Immediate	18	+	6	+	0/6
		6	40	Immediate	25	3	11	+	0/6
		6	60	Immediate	32	20	25	17	0/6
		6	80	Immediate	51	29	44	27	1/6
		6	100	Immediate	64	29	40	29	4/6
Allyl	Butyl	6	10	Not Down	+	+	+	+	0/6
		6	20	Immediate	4	+	+	+	0/6
		6	40	Immediate	9	+	7	+	0/6
		6	60	Immediate	15	5	10	+	0/6
		6	80	Immediate	36	24	25	12	0/6
		6	100	Immediate	74	45	45	23	2/6
Allyl	1-Methyl-butyl	6	20	Immediate	3	+	2	+	0/6
		6	40	Immediate	21	2	15	1	0/6
		6	60	Immediate	76	20	42	18	0/6
		6	80	Immediate	51	19	32	19	2/6
Ethyl	Allyl	6	40	Not Down	+	+	+	+	0/6
		6	60	2	76	+	10	+	0/6
		6	80	Immediate	80	14	92	9	1/6[d]
		6	100	Immediate	155	68	126	89	3/6
Ethyl	Isoamyl	6	20	Immediate	15	+	4	+	0/6
		6	30	Immediate	15	+	5	+	0/6[e]
		6	40	Immediate	26	8	13	6	0/6
		6	50	Immediate	25	7	18	4	0/6
		6	60	Immediate	47	12	16	9	2/5
Ethyl	Hexyl	6	20	Immediate	9	+	1	+	0/6
		6	30	Immediate	18	+	11	+	0/6
		6	40	Immediate	60	25	37	21	1/6
		6	50	Immediate	43	34	35	10	1/6
		6	60	Immediate	111	35	59	35	2/6
Δ-1-Cyclohexenyl	Ethyl	6	80	Immediate	91	+	+	+	0/6[f]
Ethyl	2-Ethyl-butyl	6	40	Immediate	10	+	2	+	0/6
		6	50	Immediate	13	+	3	+	0/6
		6	60	Immediate	19	2	14	+	0/6
		6	80	Immediate	30	4	18	+	0/6
		6	100	Immediate	38	19	28	+	0/6
		6	120	Immediate	65	26	44	6	1/6
		3	140	Immediate	...	...	...	...	3/3
		3	160	Immediate	...	...	...	...	3/3
Ethylthioethylene	Ethyl	6	80	Immediate	23	+	+	+	0/6
		6	100	Immediate	36	5	9	+	0/6
		6	140	Immediate	63	19	24	6	2/6
		6	160	Immediate	63	22	25	15	3/6
1-Methylbutyl	Allyl	6	20	Immediate	10	+	+	+	0/6
		6	40	Immediate	47	2	10	+	0/6
		6	60	Immediate	70	20	27	8	0/6
		12	80	Immediate	61	23	28	12	0/6
		6	90	Immediate	52	13	23	9	0/6
		6	100	Immediate	108	50	62	45	2/6
		6	110	Immediate	60	30	35	22	4/6
Secondary Butyl	Methyl	6	100	Immediate	147	65	95	25	2/6
		6	120	Immediate	190	106	131	102	1/6
		6	140	Immediate	256	162	197	152	1/6
		6	160	Immediate	317	273	289	253	2/6
1-Methylbutyl	Methyl	6	40	Immediate	30	+	7	+	0/6
		6	50	Immediate	45	14	30	+	0/6
		6	60	Immediate	66	37	50	18	0/6
		12	70	Immediate	54	29	40	13	1/12
		12	80	Immediate	73	31	39	20	2/12
		12	90	Immediate	89	53	63	44	4/12
		12	100	Immediate	65	39	51	28	6/12
β-Methylallyl	Methyl	1	20	Not down	+	+	+	+	0/1
		1	30	Not down	+	+	+	+	0/1[g]
		8	40	3	31	+	+	+	0/8[h]
		10	60	Immediate	62	+	36	+	0/10[i]

[d] Tremors on awakening
[e] Respiration irregular after injection
[f] Labored respiration on injection; convulsions on awakening
[g] Twitched ears after injection
[h] Convulsive jerks during injection
[i] Chewing movements, shakes head

TABLE II.—EFFECTS OF INTRAVENOUS INJECTIONS IN WHITE RATS OF VARIOUS SUBSTITUTED BARBITURIC ACID DERIVATIVES AS HYPNOTICS AND THE OBSERVED TOXICITY

Compound II R	Compound II R_1	No. of Animals	Dose, Mg./Kg.	Down After Injection, Min.	Duration of Loss of Various Reflexes, Min. Righting	Corneal	Ear	Tail	Killed
Ethyl	Butyl	6	10	Immediate	3	+	+	+	0/6
		6	20	Immediate	29	2	8	+	0/6
		6	40	Immediate	25	5	10	+	0/6
		6	60	Immediate	43	13	22	2	1/6
		6	80	Immediate	69	35	37	32	1/6
		6	100	Immediate	...	...	...	...	6/6
Allyl	Butyl	6	20	Immediate	17	+	6	+	0/6
		6	40	Immediate	23	10	13	10	0/6
		6	60	Immediate	37	18	19	18	0/6
		6	80	Immediate	32	10	19	14	2/6
Ethyl	Amyl	6	20	Immediate	14	1	4	+	0/6
		6	30	Immediate	25	7	10	+	0/6
		6	40	Immediate	21	10	11	+	0/6
		6	50	Immediate	41	12	22	5	0/6
		6	60	Immediate	38	33	31	14	0/6
		6	80	Immediate	94	45	58	24	2/6
		6	100	Immediate	...	...	...	...	6/6
Ethyl	Isoamyl	6	20	Immediate	21	+	8	+	0/6
		6	30	Immediate	19	+	8	+	0/6
		6	40	Immediate	31	2	16	3	0/6
		6	50	Immediate	33	5	16	5	0/6
		6	60	Immediate	45	24	26	23	2/6
Isobutyl	Allyl	10	15	Immediate	21	+	1	+	0/10[a]
		10	20	Immediate	13	+	1	+	0/10[b]
		10	25	Immediate	28	+	7	+	0/10
		6	40	Immediate	28	+	7	+	0/6
		6	60	Immediate	71	16	25	19	0/6
		6	70	Immediate	50	17	26	1	0/6
		16	80	Immediate	123	31	41	23	1/16
		6	90	Immediate	62	30	36	23	1/6
		16	100	Immediate	191	74	98	62	5/16
		6	110	Immediate	65	18	24	16	5/6
		10	115	Immediate	283	94	142	58	6/10
Propyl	Isopropyl	6	80	Immediate	45	3	8	3	0/6[e]
Propyl	Methyl	6	80	Immediate	62	+	+	+	0/6
		6	100	Immediate	81	34	40	2	0/6
		6	120	Immediate	101	39	70	3	0/6[d]
		6	140	Immediate	149	94	101	69	1/6[d]
		6	160	Immediate	184	147	161	139	1/6[d]
		6	180	Immediate	178	116	167	146	0/6[d]
		6	200	Immediate	267	176	189	138	2/6[d]
		6	220	Immediate	259	184	191	181	4/6[d]
Ethyl	Ethyl	6	60	Immediate	75	+	+	+	0/6
		6	80	Immediate	141	30	51	3	0/6
		6	100	Immediate	230	102	141	94	1/12
		6	120	Immediate	218	89	116	10	1/6
		6	140	Immediate	204	140	193	130	2/6
		6	160	Immediate	324	245	277	219	3/6
Allyl	Methyl	6	80	Immediate	126	+	43	+	0/6[e]
Amyl	Methyl	6	60	Immediate	38	+	6	+	0/6
		6	80	Immediate	38	10	22	+	0/6
		6	100	Immediate	68	26	40	11	0/6
		6	120	Immediate	76	34	39	13	3/6
Isopropyl	Butyl	6	20	Immediate	13	+	3	+	0/6[f]
		6	30	Immediate	19	+	11	+	0/6[f]
		6	40	Immediate	28	4	17	+	0/6
		6	50	Immediate	44	21	28	16	1/6[g]
		6	60	Immediate	56	28	36	21	1/6[g]
		6	80	Immediate	...	...	...	...	6/6
Amyl	Ethyl	5	60	Immediate	18	+	4	+	0/5[h]

[a] Convulsive movements—2 animals
[b] Movement, lower jaw—2 animals
[c] Convulsions
[d] Twitching and scratching on awakening
[e] Convulsions immediately after injection
[f] Convulsive twitch
[g] Convulsive twitch and scratching
[h] Convulsive jerk at injection

(*Cont'd on next page*)

TABLE II.—EFFECTS OF INTRAVENOUS INJECTIONS IN WHITE RATS OF VARIOUS SUBSTITUTED BARBITURIC ACID DERIVATIVES AS HYPNOTICS AND THE OBSERVED TOXICITY. (*Cont'd from page 235*)

Compound II R	Compound II R_1	No. of Animals	Dose Mg./Kg.	Down After Injection, Min.	Duration of Loss of Various Reflexes, Min. Righting	Corneal	Ear	Tail	Killed
Amyl	Ethyl	6	70	Immediate	26	2	7	+	2/6[e]
		6	80	Immediate	29	2	11	+	2/6[e]
		6	90	Immediate	29	10	16	3	1/6[e]
		6	100	Immediate	38	15	18	9	3/6[e]
Allyl	Isobutyl	6	30	Immediate	7	+	1	+	0/6
		6	40	Immediate	15	+	7	+	0/6
		6	50	Immediate	15	+	7	+	0/6
		6	60	Immediate	22	8	12	4	1/6
		6	70	Immediate	43	22	29	19	4/6
		6	80	Immediate	41	17	22	12	5/6
Isobutyl	Ethyl	6	40	Immediate	23	+	3	+	0/6
		6	50	Immediate	26	+	10	+	0/6
		6	60	Immediate	37	+	15	+	0/6
		6	70	Immediate	29	4	15	+	0/6[f]
		6	80	Immediate	55	26	33	13	0/6[k]
		6	90	Immediate	54	24	28	13	2/6[k]
		6	100	Immediate	74	41	54	33	5/6[k]
Butyl	Ethyl	6	60	Immediate	31	+	2	+	0/6
		6	70	Immediate	44	1	7	+	0/6
		6	80	Immediate	73	17	23	3	0/6
		6	90	Immediate	86	18	23	9	2/6
		6	100	Immediate	72	23	26	19	2/6
		6	110	Immediate	85	25	35	18	4/6
Allyl	Ethyl	6	40	Immediate	32	+	1	+	0/6
		6	50	Immediate	60	+	9	+	0/6
		6	60	Immediate	84	17	40	10	0/6
		6	70	Immediate	131	43	63	14	0/6
		6	80	Immediate	150	70	90	25	0/6
		6	90	Immediate	179	90	118	68	0/6
		6	100	Immediate	183	111	127	97	0/6
		6	110	Immediate	121	69	74	64	0/6
		6	120	Immediate	174	96	114	82	3/6
Allyl	Propyl	6	30	Immediate	15	+	5	+	0/6
		6	40	Immediate	23	2	11	+	0/6
		6	50	Immediate	27	3	13	+	0/6
		6	60	Immediate	31	14	20	2	1/6[l]
		6	70	Immediate	35	18	24	10	1/6[l]
		6	80	Immediate	38	20	25	16	1/6[l]
		6	90	Immediate	46	23	28	18	1/6[l]
		6	100	Immediate	63	43	45	41	5/6[l]
Allyl	Allyl	6	40	Immediate	39	+	3	+	0/6
		6	50	Immediate	74	9	21	+	0/6
		6	60	Immediate	117	19	38	7	0/6
		6	70	Immediate	135	34	43	22	0/6
		6	80	Immediate	131	41	51	27	1/6
		6	90	Immediate	187	43	75	24	2/6
		6	100	Immediate	210	77	170	70	5/6
Propyl	Ethyl	10	20	0.5	39	+	+	+	0/10[m]
		10	40	Immediate	81	+	5	+	0/10[n]
		10	60	Immediate	90	9	18	1	0/10[n]
		10	80	Immediate	137	12	40	2	0/10[n]
		10	100	Immediate	210	44	60	18	3/10[n]
		10	120	Immediate	156	68	96	48	7/10[n]
Compound III									
R	**R_1**								
Ethyl	Butyl	6	40	Immediate	12	+	1	+	0/6
		6	60	Immediate	14	+	9	+	0/6
		6	80	Immediate	26	5	15	3	0/6
		6	100	Immediate	43	23	30	15	0/6
		6	120	Immediate	53	29	31	24	2/6
Ethyl	Amyl	6	20	Immediate	6	+	+	+	0/6
		6	40	Immediate	16	+	6	+	0/6
		6	60	Immediate	26	11	13	+	0/6

[e] Labored respiration
[f] Drooling
[k] Constant chewing and drooling
[l] Scratching on awakening
[m] Tremors on awakening
[n] Tremors on awakening, salivation

(*Cont'd on next page*)

TABLE II.—EFFECTS OF INTRAVENOUS INJECTIONS IN WHITE RATS OF VARIOUS SUBSTITUTED BARBITURIC ACID DERIVATIVES AS HYPNOTICS AND THE OBSERVED TOXICITY. (*Cont'd from page 236*)

Compound III R	Compound III R_1	No. of Animals	Dose Mg./Kg.	Down After Injection, Min.	Duration of Loss of Various Reflexes, Min. Righting	Corneal	Ear	Tail	Killed
Ethyl	Amyl	6	80	Immediate	39	16	23	7	0/6
		6	100	Immediate	58	29	36	23	1/6
		6	120	Immediate	...	...	...	...	6/6
Allyl	Butyl	6	10	Immediate	4	+	+	+	0/6
		6	20	Immediate	12	2	8	+	0/6
		6	40	Immediate	20	6	11	+	0/6
		6	60	Immediate	24	11	13	2	0/6
		6	80	Immediate	51	26	27	23	3/6[m]
Isoamyl	Ethyl	6	60	Immediate	13	2	3	2	0/6[o]
		6	80	Immeditae	16	5	6	5	3/6[o]
Ethyl	1-Methyl-butyl	6	60	Immediate	13	+	+	+	0/6[p]
		6	80	Immediate	19	+	10	+	0/6[p]
		6	100	Immediate	25	2	13	+	0/6[p]
		6	120	Immediate	45	12	21	+	1/6[p]
		6	140	Immediate	38	11	24	10	4/6[p]
Isopropyl	Butyl	6	40	Immediate	15	+	5	+	0/6
		6	60	Immediate	20	9	13	+	0/6[p]
		6	80	Immediate	23	8	15	+	0/6[p]
		6	100	Immediate	31	19	20	13	1/6
		6	120	Immediate	37	25	31	23	4/6
Butyl	Allyl	6	80	Immediate	23	+	+	+	1/6[p]
		6	100	Immediate	20	2	4	+	4/6
Allyl	Ethyl	6	80	Immediate	40	+	9	+	0/6[q]
		6	100	Immediate	41	2	6	+	0/6[q]
		6	120	Immediate	57	13	34	+	1/6[q]
		6	140	Immediate	115	67	80	46	1/6[q]
		6	160	Immediate	68	37	40	30	2/6[q]
Methyl	Butyl	1	20	Immediate	...	...	...	...	..[r]
		1	30	Immediate	...	...	...	...	..[s]
		5	40	Immediate	...	...	...	...	..[s]
		1	60	Immediate	...	...	...	...	..[s]
		1	80	Immediate	...	...	...	...	..[s]
Allyl	Propyl	1	20	Not down	...	...	...	...	0/1
		9	30	Immediate	6	+	+	+	0/9[t]
		10	50	Immediate	11	1	4	+	0/10[u]
		10	70	Immediate	16	3	9	+	0/10[v]
		10	90	Immediate	33	14	20	9	4/10[w]
		10	110	Immediate	35	19	23	10	4/10[w]
Allyl	Isopropyl	1	20	Immediate	...	...	...	...	0/1[r]
		1	40	Immediate	...	...	...	...	0/1[r]
		1	60	.....	...	...	...	...	0/1[x]
Phenyl	Ethyl	10	40	Immediate	12	+	+	+	0/10[y]
		10	60	Immediate	28	+	2	+	0/10[z]
		10	80	Immediate	44	5	7	3	1/10[z]
		10	100	Immediate	59	6	9	4	0/10[aa]
		10	120	Immediate	63	13	17	10	0/10[aa]
		8	140	Immediate	126	18	24	15	0/8[bb]
		10	150	Immediate	122	12	19	9	3/10[bb]
		10	160	Immediate	107	13	18	10	6/10[bb]
		10	170	Immediate	146	17	23	9	9/10[bb]

[o] Convulsions, labored respiration
[p] Slight tremors on awakening
[q] Rapid respiration after injection
[r] No effect
[s] Incoordination only
[t] Grinding teeth
[u] Convulsive jerk on awakening
[v] Jerking during injection
[w] Shakes head on awakening
[x] Violent convulsions
[y] Conclusive twitch on awakening
[z] Labored respiration
[aa] Struggled during injection
[bb] Struggled during injection, lacrimation

TABLE III.—EFFECTS OF INTRAVENOUS INJECTIONS IN WHITE RATS OF VARIOUS SUBSTITUTED BARBITURIC ACID DERIVATIVES AS HYPNOTICS AND THE OBSERVED TOXICITY

Compound IV R	Compound IV R_1	No. of Animals	Dose, Mg./Kg.	Down After Injection, Min.	Duration of Loss of Various Reflexes, Min. Righting	Corneal	Ear	Tail	Killed
Ethyl	Butyl	6	30	Immediate	11	+	6	+	0/6
		6	40	Immediate	21	+	14	+	0/6
		6	50	Immediate	27	8	17	3	0/6
		6	60	Immediate	46	21	28	17	0/6
		6	70	Immediate	62	33	40	31	2/6
Allyl	Butyl	6	80	Immediate	66	34	39	30	4/6
		6	30	Immediate	7	+	3	+	0/6
		6	40	Immediate	14	+	8	+	0/6
		6	50	Immediate	18	4	11	+	0/6
		6	60	Immediate	25	9	16	5	1/6
		6	70	Immediate	...	...	...	...	6/6
		6	80	Immediate	20	10	15	+	5/6
Ethyl	Ethyl	6	80	Immediate	41	+	+	+	0/6[a]
		6	100	Immediate	84	+	18	+	0/6[a]
		6	120	Immediate	106	50	72	21	1/6[a]
		6	140	Immediate	117	64	80	36	5/6[a]
Allyl	Ethyl	6	80	Immediate	31	+	3	+	0/6[b]
		6	100	Immediate	33	18	29	+	1/6[c]
		6	120	Immediate	69	35	41	21	2/6[c]
		6	140	Immediate	80	40	64	20	4/6[c]
Compound V R	Compound V R_1								
Ethyl	Propyl	6	40	Immediate	9	+	+	+	0/6[d]
		6	60	Immediate	14	+	3	+	0/6
		6	80	Immediate	21	3	11	+	0/6
		6	100	Immediate	29	12	20	4	1/6[e]
		6	120	Immediate	35	16	21	4	0/6[e]
		6	140	Immediate	34	20	25	12	3/6
Evipal Sodium		10	30	Immediate	34	+	5	+	0/10[f]
		10	40	Immediate	28	+	6	+	0/10
		9	50	Immediate	40	1	9	+	0/9[g]
		6	60	Immediate	28	5	9	+	0/6
		6	70	Immediate	34	6	15	+	0/6
		6	80	Immediate	58	13	30	8	0/6
		6	100	Immediate	35	19	55	13	0/6
2% Solution		10	110	Immediate	53	9	19	5	1/10
5% Solution		10	110	Immediate	46	13	22	6	5/10
		10	120	Immediate	88	19	58	11	2/10
		10	130	Immediate	54	10	22	7	5/10
		6	140	Immediate	104	49	77	48	3/6
		6	160	Immediate	...	...	...	...	6/6
Amytal Sodium		6	20	Not Down	...	...	...	...	0/6
		6	40	1	92	+	+	+	0/6
		6	60	Immediate	128	2	18	+	0/6
		6	80	Immediate	192	38	85	+	1/6
		6	100	Immediate	248	76	143	+	0/6
		6	120	Immediate	292	107	176	81	1/6
		6	140	Immediate	340	180	255	98	4/6
		6	160	Immediate	428	294	330	285	4/6
Pentobarbital Sodium		6	20	Immediate	62	+	5	+	0/6
		6	30	Immediate	117	+	50	+	0/6
		6	40	Immediate	127	32	57	20	0/6
		6	60	Immediate	232	116	190	87	1/6
		6	80	Immediate	429	377	411	356	1/6
		6	100	Immediate	414	343	389	335	4/6

[a] Constant scratching, lacrimation
[b] Scratching
[c] Scratching, drooling
[d] Muscle tremors on awakening
[e] Tremors on awakening
[f] Grinding teeth—convulsions in 3
[g] Grinding teeth—1 animal

TABLE IV.—EFFECTS OF INTRAVENOUS INJECTIONS IN WHITE RATS OF VARIOUS SUBSTITUTED THIOBARBITURIC ACID DERIVATIVES AS HYPNOTICS AND THE OBSERVED TOXICITY

Compound VI R	R1	No. of Animals Used	Dose, Mg./Kg.	Down After Injection, Min.	Duration of Loss of Various Reflexes, Min. Righting	Corneal	Ear	Tail	Killed
Secondary Butyl	Ethyl	6	40	Immediate	4	+	+	+	0/6
		6	60	Immediate	14	+	5	+	0/6
		6	100	Immediate	67	+	17	+	0/6[a]
		6	120	Immediate	102	4	12	+	1/6[a]
		6	140	Immediate	135	+	22	+	0/6[a]
		6	160	Immediate	203	44	55	2	0/6[a]
		6	200	Immediate	247	17	92	57	0/6[a]
		6	240	Immediate	329	65	145	25	2/6[b]
		6	280	Immediate	480	63	250	63	5/6[b]
1-Methyl-Butyl	Ethyl	6	20	Immediate	13	+	+	+	0/6
		6	40	Immediate	32	+	9	+	0/6
		6	60	Immediate	45	3	25	7	0/6
		5	80	Immediate	55	32	31	32	0/5
		6	100	Immediate	64	51	51	51	5/6
1-Methyl-Butyl	Allyl	6	80	Immediate	40	+	19	+	0/6[c]
Compound VII R	R1								
Ethyl	Butyl	6	20	Immediate	6	+	+	+	0/6
		6	30	Immediate	10	+	4	+	0/6
		6	40	Immediate	20	6	11	+	0/6
		6	50	Immediate	24	1	11	+	0/6
		6	60	Immediate	30	9	17	+	0/6
		6	80	Immediate	115	25	34	6	1/6
		6	100	Immediate	130	34	53	16	5/6
Ethyl	Amyl	6	20	Immediate	8	+	1	+	0/6
		6	40	Immediate	23	4	13	4	0/6
		6	60	Immediate	34	15	22	8	0/6
		6	80	Immediate	113	25	45	23	1/6
		6	100	Immediate	86	3	38	13	1/6
		6	120	Immediate	...	...	...	...	6/6
Ethyl	Ethyl	6	40	Immediate	53	+	+	+	0/6
		6	60	Immediate	177	5	10	2	0/6
		6	70	Immediate	70	12	20	9	0/6
		6	80	Immediate	200	14	30	6	1/6
		6	90	Immediate	120	9	21	5	0/6
		12	100	Immediate	211	31	71	18	4/12
		6	110	Immediate	115	29	47	25	4/6
Allyl	Methyl	6	40	Immediate	48	3	10	+	0/6[d]
		6	60	Immediate	141	43	50	37	0/6
		6	80	Immediate	121	37	51	35	1/6
		6	100	Immediate	172	70	92	54	1/6
		6	120	Immediate	245	106	135	101	3/6
Amyl	Methyl	6	80	Immediate	32	+	12	+	0/6[e]
Allyl	Ethyl	6	30	Immediate	14	+	+	+	0/6
		6	40	Immediate	23	+	6	+	0/6
		6	50	Immediate	48	+	13	+	0/6
		6	60	Immediate	67	9	28	3	0/6
		6	70	Immediate	62	24	31	6	2/6
		6	80	Immediate	104	36	45	14	4/6
Propyl	Ethyl	10	20	Immediate	2	+	+	+	0/10[f]
		10	40	Immediate	24	+	3	+	0/10[g]
		10	60	Immediate	70	2	12	+	0/10[h]
Compound VIII R	R1								
Ethyl	1-Methyl-Butyl	6	80	Immediate	24	+	13	+	0/6[i]
		6	100	Immediate	43	+	20	+	0/6[i]
Ethyl	Amyl	6	20	Immediate	2	+	+	+	0/6
		6	40	Immediate	12	+	7	+	0/6
		6	60	Immediate	21	+	11	+	0/6

[a] Muscle tremors after injection
[b] Hiccough respiration
[c] Convulsions during injection and for 3 min. Violent tremors on awakening
[d] Sneezing, shaking head on awakening
[e] Convulsions after injection
[f] Licking
[g] Convulsive twitching
[h] Convulsive twitching, shaking
[i] Muscle tremors

(*Cont'd on next page*)

TABLE IV.—EFFECTS OF INTRAVENOUS INJECTIONS IN WHITE RATS OF VARIOUS SUBSTITUTED THIOBARBITURIC ACID DERIVATIVES AS HYPNOTICS AND THE OBSERVED TOXICITY. (*Cont'd from page 239*)

Compound VIII R	R1	No. of Animals Used	Dose Mg./Kg.	Down After Injection, Min.	Duration of Loss of Various Reflexes, Min. Righting	Corneal	Ear	Tail	Killed
Ethyl	Amyl	6	80	Immediate	35	9	21	+	0/6
		6	100	Immediate	62	27	41	8	0/6[j]
		6	120	Immediate	63	20	43	13	1/6
		6	140	Immediate	74	31	59	25	3/6
Ethyl	Butyl	6	20	Immediate	3	+	+	+	0/6
		6	40	Immediate	9	+	3	+	0/6
		6	60	Immediate	30	3	12	+	0/6
		6	80	Immediate	50	14	17	+	0/6
		6	100	Immediate	58	23	36	20	0/6[j]
		6	120	Immediate	100	40	72	14	1/6
		6	140	Immediate	107	54	65	41	3/6
Allyl	Butyl	10	15	Immediate	5	+	+	+	0/10
		10	20	Immediate	6	+	3	+	0/10
		10	25	Immediate	12	+	+	+	0/10
		12	40	Immediate	20	1	9	1	0/12
		12	60	Immediate	24	8	15	5	0/12
		12	80	Immediate	32	15	21	10	0/12
		22	100	Immediate	44	19	26	13	22/2
		10	110	Immediate	37	12	19	7	0/10
		10	120	Immediate	53	19	30	7	5/10
		10	130	Immediate	54	14	28	10	7/10
Butyl	Allyl	2	80	Immediate	13	+	+	+	0/2[k]
Isopropyl	Butyl	6	20	Immediate	8	+	2	+	0/6[j]
		6	40	Immediate	21	+	11	+	0/6[j]
		6	60	Immediate	35	2	16	+	0/6[j]
		6	80	Immediate	31	11	17	5	0/6[j]
		6	100	Immediate	48	20	26	7	1/6[j]
		6	120	Immediate	61	25	39	16	2/6[j]
Isoamyl	Ethyl	2	80	Not down	...	...	...	...	0/2[m]
Allyl	Ethyl	6	80	Immediate	33	+	8	+	0/6[n]
Compound IX R	R1								
Ethyl	Butyl	6	10	Immediate	11	+	3	+	0/6
		6	20	Immediate	29	9	14	+	0/6
		6	40	Immediate	107	12	51	+	1/6
		6	60	Immediate	109	31	104	16	2/6
		6	80	Immediate	...	...	...	...	6/6
Compound X R	R1								
Ethyl	Ethyl	6	40	Immediate	110	37	55	8	1/6[o]
		6	60	Immediate	192	130	122	39	3/6[p]
		6	80	Immediate	...	...	...	...	6/6
Compound XI R	R1								
Ethyl	Ethyl	6	30	Immediate	20	+	3	+	0/6[q]
		6	40	Immediate	78	+	26	+	0/6[r]
		6	50	Immediate	119	9	28	+	1/6[s]
		6	60	Immediate	186	33	61	7	3/6
		6	70	Immediate	...	...	...	...	6/6
		6	80	Immediate	269	52	168	50	5/6
Allyl	Butyl	6	30	Immediate	18	+	6	+	0/6
		6	40	Immediate	21	+	11	+	0/6
		6	50	Immediate	31	+	11	+	0/6
		6	60	Immediate	33	5	15	3	0/6
		6	70	Immediate	...	...	...	...	6/6
Ethyl	Butyl	6	20	Immediate	19	+	5	+	0/6
		6	30	Immediate	62	10	31	3	1/6

[j] Tremors on awakening
[k] Violent convulsion during and after injection
[l] Slight tremors on awakening
[m] Violent convulsion during and after injection, strychnine-like
[n] Convulsions after injection, labored respiration
[o] Convulsive respiration
[p] Convulsive movements on awakening
[q] Convulsion
[r] Jerking and drooling
[s] Convulsion and drooling

(*Cont'd on next page*)

TABLE IV.—EFFECTS OF INTRAVENOUS INJECTIONS IN WHITE RATS OF VARIOUS SUBSTITUTED THIOBARBITURIC ACID DERIVATIVES AS HYPNOTICS AND THE OBSERVED TOXICITY. (*Cont'd from page 240*)

Compound XI R	Compound XI R_1	No. of Animals Used	Dose Mg./Kg.	Down After Injection, Min.	Duration of Loss of Various Reflexes, Min. Righting	Corneal	Ear	Tail	Killed
Ethyl	Butyl	6	40	Immediate	73	29	44	14	0/6
		6	50	Immediate	...	...	...	...	6/6
		6	60	Immediate	...	...	...	...	6/6
Allyl	Ethyl	6	40	Immediate	21	+	1	+	0/6[f]
		6	50	Immediate	23	+	8	+	0/6[f]
		6	60	Immediate	37	3	17	+	0/6[f]
		6	70	Immediate	42	4	19	+	0/6[f]
		6	80	Immediate	54	10	30	3	1/6[f]
		6	100	Immediate	66	11	32	6	3/6[f]
Pentothal Sodium		10	15	Immediate	27	+	2	+	0/10[m]
		10	20	Immediate	39	+	11	+	0/10
		10	25	Immediate	41	+	9	+	0/10[r]
		12	40	Immediate	226	41	113	27	1/12
		10	50	Immediate	312	55	202	33	6/10
		22	60	Immediate	366	118	253	65	17/22
		6	80	Immediate	...	...	...	...	6/6

[f] Convulsions on injection and awakening
[m] Shakes head, moves lower jaw
[r] Respiratory convulsions

TABLE V.—OBSERVED TOXICITY IN WHITE RATS FOLLOWING ORAL ADMINISTRATION OF VARIOUS SUBSTITUTED BARBITURIC AND THIOBARBITURIC ACID DERIVATIVES

Compound	R	R_1	LD_{50}
I	1-Methylbutyl	Ethyl	366
	Isobutyl	Methyl	505
	1-Methylbutyl	Allyl	702
	1-Methylbutyl	Methyl	364
II	Ethyl	Butyl	582
	Allyl	Butyl	639
	Isobutyl	Allyl	417
	Allyl	Ethyl	356
	Allyl	Allyl	346
VII	Ethyl	Ethyl	772
VIII	Allyl	Butyl	1453
Pentobarbital Sodium			99

Chronic Toxicity—Rat.—Seven of the new derivatives were administered orally to rats in chronic studies. Doses of the compounds representing 25% of the LD_{50} were given twice daily for fourteen consecutive days and the animals then sacrificed for pathological examination. Fifteen animals were dosed in this manner for each of the compounds investigated. The pathologic findings, summarized in Table VI, appear to indicate the absence of gross or irreversible changes in the tissues and organs examined.

Duration of Effect and Toxicity—Mouse.—In this study male, white, Swiss-strain mice, weighing between 20 and 35 Gm., were injected intraperitoneally with selected doses of the sodium barbiturates in aqueous solution. The values shown in the tables are, however, calculated as quantities of free acid. Sodium Amytal, Sodium Pentobarbital, and Sodium Evipal were used as comparative test agents. The observations recorded in Table VII include (*a*) the mean hypnotic dose, (*b*) duration of sleep as indicated by the loss and reappearance of the righting reflex, (*c*) the time required to regain normal movement, and (*d*) the acute toxicity.

The mean hypnotic dose, defined as the dose producing brief loss of righting reflex in 50% of the animals, was determined by injecting groups of 10 animals with 0.5% to 1.0% solutions at 3 dosage levels. Data for the estimation of the acute toxicities were obtained by injecting, at 5 to 10 predetermined dosage levels, 2% solutions of the sodium barbiturates. With the results thus obtained values of the LD_{50} were computed by the use of Kärber's method. Having established the LD_{50} for each compound, it was possible to compare the effects of the barbiturates by injecting in each instance a single dose equivalent to 60% of the LD_{50} (see Table VII). For this purpose 10 animals were injected with each compound.

The variation in duration of action and toxicity among the barbiturates observed in the mouse was comparable to that seen in the rat.

TABLE VI.—EFFECTS OF CHRONIC ORAL ADMINISTRATION IN WHITE RATS OF VARIOUS SUBSTITUTED BARBITURIC AND THIOBARBITURIC ACID DERIVATIVES

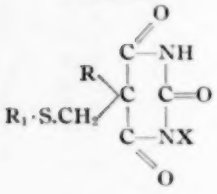
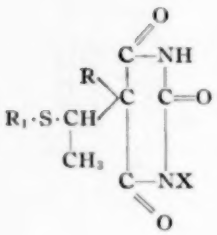
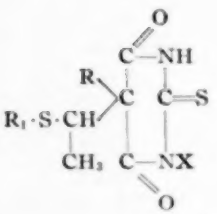
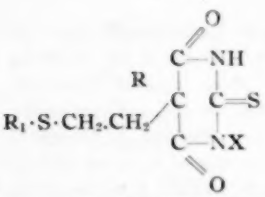
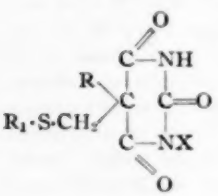
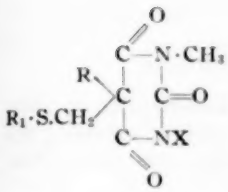
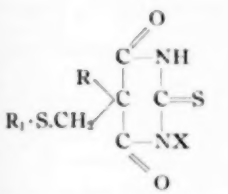
Compound	R	R_1	No. of Animals	Dose, 25% LD_{50}, Mg./Kg.	Consecutive Days Dosed	Pathologic Findings
	1-Methylbutyl	Allyl	15	176	14	Acute glomerulonephritis Fatty metamorphosis of liver
	Ethyl	Butyl	15	146	14	Normal
	Allyl	Butyl	15	160	14	Very mild acute glomerulonephritis Very mild metamorphosis of liver
	Isobutyl	Allyl	15	104	14	Normal
	Allyl	Ethyl	15	89	14	Normal
	Ethyl	Ethyl	15	193	14	One kidney showed localized inflammatory lesion in cortex Lesion in healing stage and of many days duration
	Allyl	Butyl	15	363	14	Very mild acute glomerulonephritis Mild fatty metamorphosis of liver

TABLE VII.—EFFECTS OF INTRAPERITONEAL INJECTIONS IN WHITE MICE OF VARIOUS SUBSTITUTED BARBITURIC AND THIOBARBITURIC ACID DERIVATIVES AS HYPNOTICS AND THE OBSERVED TOXICITY

Compound	R	R_1	*MHD*, Mg./Kg.	LD_{50}, Mg./Kg.	Duration of Anesthesia, 60% LD_{50}, Min.	Incoordination, 60% LD_{50}, Min.
	Methyl	Ethyl	175	750	330	0
	Methyl	Butyl	90	379	150	13
	Isobutyl	Methyl	65	354	108	24
	Allyl	Propyl	30	284	117	12
	Allyl	Butyl	20	194	123	14
	Isopropyl	Butyl	30	252	38	10
	Butyl	Butyl	30	268	95	20
	Isobutyl	Butyl	40	300	78	16
	Secondary Butyl	Butyl	40	244	69	30
	Ethyl	Ethyl	75	480	143	13
	Ethyl	Propyl	60	318	59	13
	Ethyl	Isopropyl	85	415	139	14
	Ethyl	Butyl	35	213	66	7
	Ethyl	Amyl	20	191	75	19
	Propyl	Ethyl	50	397	150	12
	Isopropyl	Ethyl	75	286	86	13
	Allyl	Ethyl	60	380	164	14
	Butyl	Ethyl	85	379	74	10
	Isobutyl	Ethyl	50	335	81	8

(Cont'd on next page)

TABLE VII.—EFFECTS OF INTRAPERITONEAL INJECTIONS IN WHITE MICE OF VARIOUS SUBSTITUTED BARBITURIC AND THIOBARBITURIC ACID DERIVATIVES AS HYPNOTICS AND THE OBSERVED TOXICITY
(*Cont'd from page 242*)

Compound	R	R_1	*MHD* Mg./Kg.	LD_{50} Mg./Kg.	Duration of Anesthesia 60% LD_{50}, Min.	Incoordination 60% LD_{50}, Min.
	Secondary Butyl	Ethyl	40	270	61	11
	1-Methylbutyl	Ethyl	45	213	35	8
	Isoamyl	Ethyl	90	285	8	6
	Propyl	Butyl	30	272	76	15
	Ethyl	Secondary Butyl	...	231	...	..
	Ethyl	Isobutyl	...	277	...	..
	Methallyl	Ethyl	...	250	...	..
	Ethyl	Tertiary Butyl	...	256	...	..
	Methyl	Ethyl	...	446	166	12
	Methyl	Butyl	50	305	116	13
	Propyl	Ethyl	...	541	168	30
	Isobutyl	Ethyl	...	420	Convulsive	..
	Ethyl	Ethyl	...	350	Convulsive	..
	Isopropyl	Ethyl	75	350	Convulsive	..
	Ethyl	Isopropyl	...	100–200	...	..
	Ethyl	Propyl	75	239	41	13
	Ethyl	Ethyl	...	402	190	21
	Secondary Butyl	Ethyl	75	375	...	..
	1-Methylbutyl	Ethyl	75	350	...	..
	Isobutyl	Methyl	75	314	Convulsive	..
	Isobutyl	Ethyl	75	360	...	..
Evipal Sodium	....	....	...	220	35	16
Pentobaribtal Sodium	....	....	...	155	120	7
Amytal Sodium	....	....	...	260	99	7

SUMMARY

1. A group of new barbituric and thiobarbituric acids have been examined experimentally by intravenous injection in the rat and intraperitoneal injection in the mouse.

2. Data have been compiled on (*a*) the time of onset of hypnosis, (*b*) duration of action as revealed by various criteria, and (*c*) toxicity.

3. Several of the new derivatives have been administered orally to rats and the tolerance to repeated doses determined.

4. The observations permit the conclusion that the duration of anesthetic or hypnotic effect of the new compounds decreased as the molecular weight increased. Generally the series containing $R'SCH(CH_3)$— as a substituent were more active compounds as revealed by the acute toxicity. In an extensive series of compounds as described in this paper there are notable exceptions to any generalization regarding the relationship of chemical structure to activity.

REFERENCES

(1) Walter, L. A., Goodson, L. H., and Fosbinder, R. J., *J. Am. Chem. Soc.*, **67**, 655, 657, 659, 661(1945).
(2) Barlow, O. W., Climenko, D. R., and Homburger, E., *Proc. Soc. Exptl. Biol. Med.*, **49**, 11(1942).
(3) Kinsey, V. Everett, THIS JOURNAL, **29**, 387(1940).
(4) Kärber, *Arch. Expt. Path. u. Pharm.*, **162**, 480(1931); through Burn, J. H., "Biological Standardization," Oxford Univ. Press, London, 1937.

The Anesthetic Effects and Toxicities of Some New Barbituric Acid Derivatives Administered to Rabbits. II*

By WILLIAM H. HUNT† RUSSEL J. FOSBINDER, and O. W. BARLOW

IN A PREVIOUS paper (1) we have described the results obtained with a new series of barbituric acid derivatives following intravenous and intraperitoneal administration to the rat and mouse, respectively.

Continuing the experimental study of the new compounds reported by Walter, Goodson, and Fosbinder (2) we present herein pertinent data relative to the hypnotic action and toxicities of the barbituric acid derivatives administered intravenously to rabbits. Observations on the rate of detoxification of selected compounds are also included.

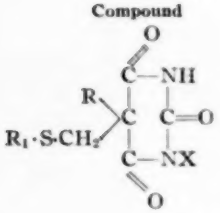
* Received Dec. 17, 1945, from the Research Laboratories of the Maltbie Chemical Co., Newark, N. J., and the Winthrop Chemical Co., Rensselaer, N. Y.

† Present address: Marvin R. Thompson Laboratories, Stamford, Conn.

EXPERIMENTAL

Duration of Effect and Toxicity.—Adult male, white, New Zealand-strain rabbits, averaging 2.5 Kg. in weight, were employed in this study. Five

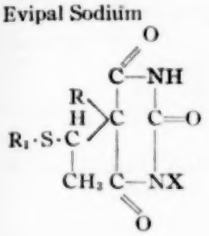
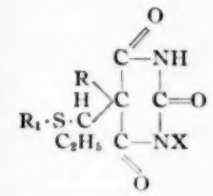
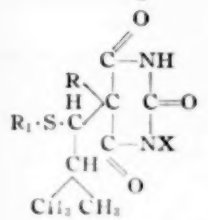
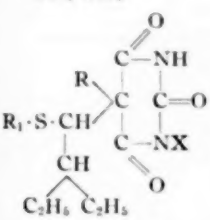
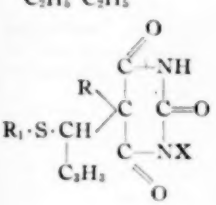
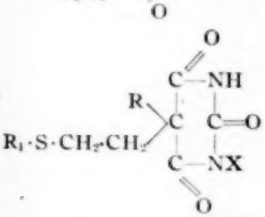
TABLE I.—DURATION OF HYPNOTIC EFFECTS AND OBSERVED TOXICITIES OF VARIOUS SUBSTITUTED BARBITURIC ACID DERIVATIVES INJECTED INTRAVENOUSLY IN RABBITS



R	R_1	*MHD*, Mg./Kg.	*LD*, Mg./Kg.	Duration Anesthesia, 60% *LD*, Min.	Incoordination, 60% *LD*, Min.	Remarks
Allyl	Propyl	25	73	37	66	
Allyl	Butyl	10	47	39	24	
Isopropyl	Butyl	15	53	43	28	
Butyl	Butyl	20	60	30	22	
Isobutyl	Butyl	20	62	25	31	
Secondary Butyl	Butyl	10	44	27	26	
Ethyl	Ethyl	50	211	121	74	
Ethyl	Propyl	40	124	33	51	
Ethyl	Butyl	25	78	36	26	
Ethyl	Isobutyl	40	104	39	37	Locomotion of forelegs
Ethyl	Tertiary Butyl	..	162	...	..	Locomotion of forelegs
Ethyl	Amyl	15	53	25	19	
Ethyl	1-Methylbutyl	25	73	62	11	Locomotion of forelegs
Ethyl	Isoamyl	20	61	37	12	
Ethyl	Hexyl	15	43	15	13	
Ethyl	2-Ethylbutyl	20	65	22	14	
Ethyl	Cyclohexyl	..	...	...	..	Violent convulsions
Propyl	Ethyl	40	171	120	97	
Isopropyl	Ethyl	30	106	92	45	Sneezing on awakening
Allyl	Ethyl	30	158	106	28	
Isobutyl	Ethyl	35	115	72	46	
Secondary Butyl	Ethyl	27	101	67	44	
1-Methylbutyl	Ethyl	22	70	37	20	Locomotion of forelegs
Isoamyl	Ethyl	43	124	28	16	
Cyclohexenyl	Ethyl	30	93	59	38	Locomotion of legs
Hexyl	Ethyl	20	44	Very brief		
Ethyl	Allyl	45	156	117	41	Locomotion of legs, convulsive action
Methallyl	Ethyl	..	...	...	..	Locomotion of legs, convulsive action
Propyl	Butyl	20	69	48	39	

(*Cont'd on next page*)

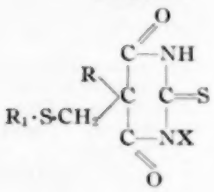
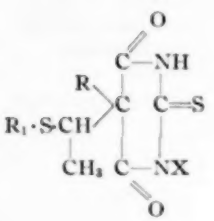
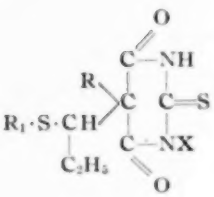
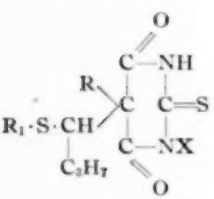
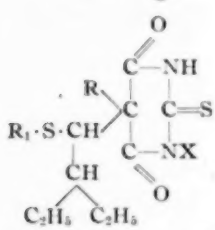
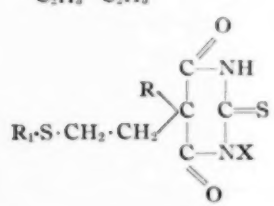
TABLE I.—DURATION OF HYPNOTIC EFFECTS AND OBSERVED TOXICITIES OF VARIOUS SUBSTITUTED BARBITURIC ACID DERIVATIVES INJECTED INTRAVENOUSLY IN RABBITS. (*Cont'd from page 244*)

Compound	R	R_1	MHD_{50}, Mg./Kg.	LD_{50}, Mg./Kg.	Duration Anesthesia, 60% LD_{50}, Min.	Incoordination, 60% LD_{50}, Min.	Remarks
Pentobarbital Sodium			..	45	58	24	
Amytal Sodium			..	75	89	48	
Evipal Sodium			15	80	19	13	
	Propyl	Isobutyl	35	116	45	19	Convulsive tremors
	Isobutyl	Allyl	..	46	28[a]	..	Locomotion of legs, convulsive action
	Allyl	Butyl	10	35	34	21	
	Ethyl	Butyl	15	55	34	21	Locomotion of legs, convulsive twitch
	Propyl	Butyl	20	58	35	32	Locomotion of legs
	Ethyl	Amyl	10	36	28	9	
	Ethyl	Isoamyl	15	45	41	10	Locomotion of legs, convulsive action
	Hexyl	Ethyl	20	45	12	17	Locomotion of legs
	Methyl	Cyclohexyl	..	...	...	..	Convulsions and nystagmus
	Propyl	Methyl	..	...	...	..	M. H. D. between 61–124 mg./Kg.
	Ethyl	Butyl	20	65	52	21	Convulsive action
	Ethyl	Isopropyl	..	10	...	..	Strychnine-like death
	Ethyl	Ethyl	30	44	12	14	
	Ethyl	Ethyl	54	65	...	..	
	Ethyl	Ethyl	..	58	...	..	Convulsive action
	Ethyl	Butyl	25	73	24	8	

[a] Dose, 50% LD_{50}.

and 10% solutions of the sodium barbiturates were used throughout the experiments, permitting the administration of fairly uniform volumes in each instance. The injections were made into the marginal ear vein at a constant rate of 2.0 cc.. per minute. In this study, Sodium Pentobarbital, Sodium Pentothal, and Sodium Evipal were used as reference compounds. The criteria employed for the determination of the mean hypnotic dose, duration of anesthesia, and the incoordination interval were roughly similar to those used by Werner, Pratt, and Tatum (3). The acute toxicities of the barbiturates

TABLE II.—DURATION OF HYPNOTIC EFFECTS AND OBSERVED TOXICITIES OF VARIOUS SUBSTITUTED THIOBARBITURIC ACID DERIVATIVES INJECTED INTRAVENOUSLY IN RABBITS

Compound	R	R_1	MHD_{50}, Mg./Kg.	LD_{50}, Mg./Kg.	Duration Anesthesia, 60% $I.D_{50}$, Min.	Incoordination, 60% LD_{50}, Min.	Remarks
	Isobutyl	Methyl	..	110	50	40	
	Ethyl	Isoamyl	20	61	12	5	Very irritant on injection
	Isobutyl	Ethyl	30	129	45	39	
	Secondary Butyl	Ethyl	25	82	48	23	
	1-Methyl-butyl	Ethyl	20	56	16	12	
	Butyl	Butyl	..	...	..	..	Convulsions
	Propyl	Methyl	..	103	..	..	Very long duration
	Hexyl	Ethyl	25	58	8	4	Convulsions
	Ethyl	Butyl	15	57	23	16	Locomotion of legs
	Propyl	Butyl	20	58	21	21	Convulsive twitches
	Ethyl	Amyl	15	45	14	4	
	Ethyl	Isoamyl	20	51	15	6	
	Methyl	Cyclohexyl	..	...	..	..	Violent Convulsions, nystagmus
	Allyl	Butyl	15	40	22	11	Locomotion of legs
	Isobutyl	Allyl	20	73	30	33	Convulsive irritant on injection
	Ethyl	Isopropyl	..	...	..	..	Strychnine-like death
	Ethyl	Butyl	15	40	18	9	
	Ethyl	Ethyl	10	32	9	4	Convulsive action
	Ethyl	Ethyl	..	...	..	..	Cyanosis, mercaptan breath, appears to produce methemoglobin
	Allyl	Butyl	20	60	..	..	

were estimated by the application of Behrens' method (4). For the determination of the MHD_{50} (median hypnotic dose) and the LD_{50}, 10 animals were injected at each dosage level. Those animals used more than once were given a rest period sufficient for the clearance of residual barbiturate. A direct comparison of the duration of anesthesia produced by the new barbiturates and the reference compounds was obtained by injecting groups of 10 animals with a dose corresponding to 60% of the estimated LD_{50}.

The results are summarized in Tables I and II wherein the various compounds are listed and data are given on the MHD_{50}, the LD_{50}, duration of anesthesia produced by injection of a dose equivalent to 60 per cent of the LD_{50}, and the incoordination time at this dosage level. Side-effects observed following intravenous administration are included under the heading "Remarks." Of particular interest was the convulsant action of 5-Ethyl,5-α-isopropylthiopropyl barbiturate sodium which is structurally related to Sodium 5-ethyl,5-1,3-dimethylbutyl barbiturate studied by Swanson and Chen (5) who reported the barbiturate was a convulsant in

TABLE III.—DETOXIFICATION OF VARIOUS SUBSTITUTED BARBITURIC AND THIOBARBITURIC ACID DERIVATIVES ADMINISTERED INTRAVENOUSLY TO RABBITS

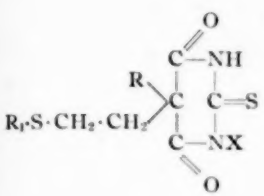
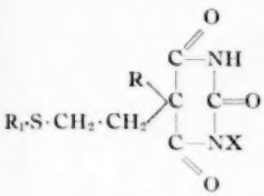
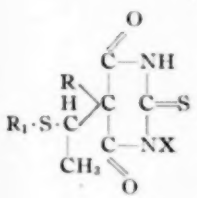
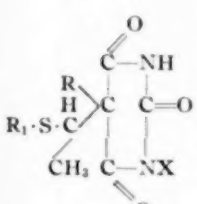
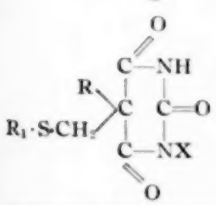
Compound	R	R_1	No. of Animals	Single Dose, Mg./Kg.	Per Cent, LD_{50}	Time Interval, Min.	No. of Doses	Average	Total Dose, % LD_{50}	Average, % LD_{50}
Evipal Sodium			10	27	33	15	4–7	5.1	135–236	172.3
			10	40	50	15	1–4	2.9	50–200	145.0
			5	40	50	23	2–4	3.0	100–200	150.0
Pentothal Sodium			10	13	33	15	5–10	7.1	138–325	228.5
			10	20	50	15	3–5	4.1	150–250	205.0
			5	20	50	23	3–5	3.8	150–250	190.0
	Allyl	Butyl	10	20	33	15	9–17	12.7	300–567	380.3
			10	30	50	15	3–11	7.5	150–550	375.0
			5	30	50	23	10	10.0	No Deaths	
	Allyl	Butyl	5	30	50	15	3–5	3.4	150–250	170.0
	Isopropyl	Butyl	5	30	50	15	3–5	3.6	150–250	180.0
	Ethyl	Butyl	5	37	50	23	3–4	3.4	150–200	170.0
	Ethyl	Amyl	5	30	50	15	3–5	3.8	150–250	190.0
	Ethyl	Ethyl	5	17	33	15	7–12	9.6	238–408	326.0
			5	25	50	15	4–6	5.2	200–300	260.0
			5	25	50	23	5–8	6.6	250–400	330.0
	Isobutyl	Allyl	10	16	33	15	4–9	6.8	133–313	231.2
			10	24	50	15	2–5	3.8	100–250	190.0
			5	24	50	23	3–4	4.2	150–200	180.0
	Isopropyl	Butyl	5	27	50	15	3–4	3.6	150–200	180.0
	Secondary Butyl	Butyl	5	22	50	15	4–9	6.0	200–450	300.0

TABLE IV.—DETOXIFICATION OF VARIOUS SUBSTITUTED BARBITURIC AND THIOBARBITURIC ACID DERIVATIVES ADMINISTERED INTRAVENOUSLY TO RABBITS

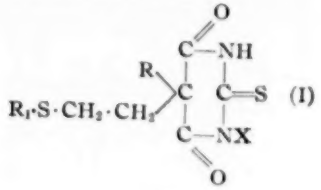
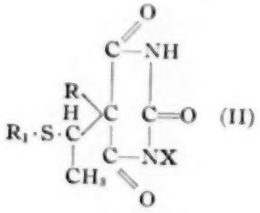
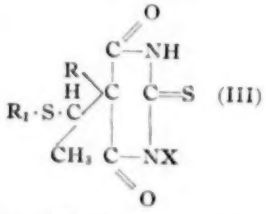
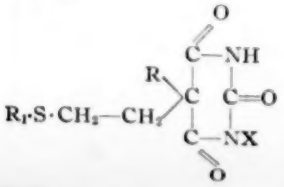
Compound	R	R_1	No. of Animals	Dose Level	No. of Animals Dead After 15-Min. Intervals — No. of Intervals																Survivors
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Evipal Sodium			10	33% LD_{50}	..	..	..	3	4	2	1	..	..	..	..	..	..	..	..	..	..	
Pentothal Sodium			10	33% LD_{50}	..	..	..	..	1	2	4	2	..	1	..	..	..	..	..	..	..	
 (I)	Allyl	Butyl	10	33% LD_{50}	..	..	..	..	..	..	..	..	1	..	2	1	3	..	1	..	1	1
 (II)	Isobutyl	Allyl	10	33% LD_{50}	..	..	..	1	1	3	1	2	2	..	..	..	..	..	..	..	..	
 (III)	Ethyl	Ethyl	5	33% LD_{50}	..	..	..	..	..	..	1	1	2	..	..	2	..	..	..	..	..	
Evipal Sodium	.....	...	10	50% LD_{50}	1	..	8	1	..	..	..	..	..	..	..	..	..	..	..	..	..	
Pentothal Sodium	.....	...	10	50% LD_{50}	..	..	2	5	3	..	..	..	..	..	..	..	..	..	..	..	..	
(I)	Allyl	Butyl	10	50% LD_{50}	..	..	1	1	..	..	3	1	2	1	1	..	..	..	..	..	..	
(II)	Isobutyl	Allyl	10	50% LD_{50}	..	1	2	5	2	..	..	..	..	..	..	..	..	..	..	..	..	
(III)	Ethyl	Ethyl	5	50% LD_{50}	..	..	..	1	2	2	..	..	..	..	..	..	..	..	..	..	..	..
 (IV)	Ethyl	Amyl	5	50% LD_{50}	..	..	2	2	1	..	..	..	..	..	..	..	..	..	..	..	..	
	Isopropyl	Butyl	5	50% LD_{50}	..	..	3	1	1	..	..	..	..	..	..	..	..	..	..	..	..	
	Allyl	Butyl	5	50% LD_{50}	..	..	4	..	1	..	..	..	..	..	..	..	..	..	..	..	..	
	Ethyl	Butyl	5	50% LD_{50}	..	..	..	3	1	1	..	..	..	..	..	..	..	..	..	..	..	

TABLE IV.—DETOXIFICATION OF VARIOUS SUBSTITUTED BARBITURIC AND THIOBARBITURIC ACID DERIVATIVES ADMINISTERED INTRAVENOUSLY TO RABBITS

(*Cont'd from page 248*)

Compound	R	R_1	No. of Animals	Dose Level	No. of Animals Dead After 15-Min. Intervals — No. of Intervals															Survivors
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
	I (propyl	Butyl	5	50% LD_{50}	..	..	2	3	..	..	..	..	1	..	..	..	..	..	..	..	..	
	Secondary	Butyl	5	50% LD_{50}	..	..	..	1	1	2	..	..	..	..	..	..	..	..	..	..	..	
	Butyl																					
Evipal Sodium			5[a]	50% LD_{50}	..	2	1	2	..	..	..	..	..	..	..	..	..	..	..	..	..	
Pentothal Sodium			5[a]	50% LD_{50}	..	..	2	2	1	..	..	..	..	..	..	..	..	..	..	..	5	
(I)			5[a]	50% LD_{50}	..	..	..	3	..	..	..	..	..	..	..	..	..	..	..	..	..	
(II)			5[a]	50% LD_{50}	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
(III)			5[a]	50% LD_{50}	..	..	..	..	2	..	1	2	..	..	..	..	..	..	..	..	..	
(IV)			5[a]	50% LD_{50}	..	..	3	2	..	..	..	..	..	..	..	..	..	..	..	..	..	

[a] Injected at 23-min. intervals.

warm-blooded animals when given in relatively low dosage. In this instance the substitution of —CH_2— by —S— apparently did not alter the pharmacological action.

All of the compounds appeared to depress the respiratory rate to a varying degree and in general the effect was dependent on the size of the dose administered. A pharmacodynamic study of this depression was not attempted.

As a part of a more detailed study of several barbiturates, selected from the group of compounds examined, the rate of a detoxification following intravenous administration was determined using a procedure similar to that described by Hazleton, Koppanyi, and Linegar (6); Pentothal Sodium and Evipal Sodium were used as control agents. In separate groups of animals, doses equivalent to 33% and 50% of the LD_{50} were given at fifteen-minute intervals until death ensued, while another group received doses of 50% of the LD_{50} at twenty-three-minute intervals. The data shown in Tables III and IV summarize the results thus obtained.

SUMMARY

1. A group of new barbituric and thiobarbituric acids have been studied by intravenous injection in rabbits. The median hypnotic dose, acute toxicities, and duration of action at a uniform dosage level, have been determined and described.

2. The rate of detoxification on intravenous administration has been determined for several of the compounds. Sodium 5-allyl-5-(α-butylthioethyl) thiobarbiturate and Sodium 5-ethyl-5-(α-ethylthioethyl) thiobarbiturate were rapidly detoxified by rabbits.

3. When the new barbiturates were administered intravenously it was observed that the duration of hypnotic effect decreased and the toxicity increased as the molecular weight of the compounds increased. Derivatives comprising the RSCH— series were generally more toxic than those in the analogous $RSCH_2$— series.

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The Influence of Physiologically Active Compounds and Their Inactive Homologs on Phase Boundary Potentials*

By CHARLES P. DILLON,† GAYLORD B. ESTABROOK, and WALTER H. HARTUNG

Employing a "model cell," as described by Beutner, the change produced in electromotive force by propadrine and three homologs and by choline and two homologs was determined. The results show that it is not yet possible to correlate change in E. M. F. with physiological activity in homologous series; there is an approximate parallelism between effect on E. M. F. and relative toxicity.

BEUTNER (1) suggests that many drugs, particularly those which are active in small doses, produce their physiological effect by altering the electromotive forces which normally exist between the heterogeneous components of the protoplasm. He has elaborated on this hypothesis and produced experimental data in its support (2).

For example, a "model cell" was set up as follows:

Hg^-	KCl solution saturated with HgCl	Nitrobenzene and 10% oleic acid	Physiological saline with 0.02% sodium oleate. Drug added here	KCl saturated with HgCl	Hg^+

The normal electromotive force of this cell, without alkaloid or drug, is approximately 85 millivolts. The addition of drug or chemical to the physiological saline solution at the point indicated reduces the E. M. F. Typical results are assembled in Table I.

Acetylcholine at a concentration of $1:3 \times 10^{-4}$ produces a negative potential of 200 mv. and at $1:1 \times 10^{-8}$, a negative potential of 5 mg. (4). Paredrine at a concentration of $1:5 \times 10^{-4}$, in a cell using tricaproin as the oil phase, reduced the potential 26 mv. (5).

The implications of the hypothesis are manifold and intriguing. It will be observed, however, even when all the data are considered, that Beutner has confined his studies to compounds known to be physiologically active and generally useful. This raises the question as to whether or not these electrical properties are characteristic only for the active members of a homologous series of compounds. If this should prove true, then it is reasonable to expect that biological activity could be predicted for an unknown compound merely by measuring the change it produces in the E. M. F. of a model cell; the character of the physiological properties may then be determined by subsequent *in vivo* examination. If so, such measurements should provide an invaluable means by which the chemist and the pharmacologist, in their search for new drugs, could narrow their investigations by prompt elimination of unpromising compounds.

Two series, the active members of which have been studied by Beutner, are available, viz., the pressor amines derived structurally from β-phenylethylamine and the quaternary ammonium compounds related to choline.

An extensive series of compounds struc-

TABLE I.—EFFECT OF ALKALOIDS AND THEIR SALTS ON E. M. F. OF "MODEL CELL" (3)

Substance	Concentration × 10^8	Decrease in E. M. F., Mv.
Pilocarpine	1	5
	10	33
	50	57
Atropine sulfate	1	4
	10	25
	50	57
Strychnine sulfate	1	4
	5	18
	10	31
Quinine hydrochloride	1	1
	10	11
Morphine sulfate	2	6
	5	11
Ephedrine sulfate	1	6
Cocaine hydrochloride	1	3

* Received April 29, 1946, from the Research Laboratories, School of Pharmacy, University of Maryland, Baltimore, Md.

† Present address: St. Joseph's College, Mountain View, Calif.

turally related to epinephrine, ephedrine, and benzedrine was available from previous studies in correlating the relationship between chemical structure and physiological activity (6).

A second group of related compounds is found in the choline series, members of which were placed at our disposal by Merck & Company, through the courtesy of Drs. Bastedo and Major. Choline and β-methylcholine are active, and the relative inertness and greater toxicity of "triethylcholine" are known (7).

It is obvious that any model cell can at best only approximate biological conditions, not duplicate them. Hence, negative results are not conclusive; rather they add complexity to the hypothesis. For instance, is it perhaps the products resulting from the reaction of the drug with the protoplasm which causes the electromotive variation? And physiologically inactive homologs or isomers do not give rise to products which produce a change in E. M. F.?

EXPERIMENTAL

The cell used in these experiments was similar to that described by Beutner (1). The electrodes were of the usual calomel type filled with saturated potassium chloride solution. To one of the electrodes was sealed a hook-shaped tube, at the lower end of which was fused a cup of 2- or 3-ml. capacity. This cup held the "oil" which set up the phase boundary potential.

For measuring the changes in potential difference a Beckman *p*H meter, laboratory model G, was used. This type of potential measuring apparatus was applicable, since the cell had very high internal resistance. This instrument gave measurements reproducible to 5 mv. Readings were taken with the poles connected one way, and then repeated with the poles reversed.

In making a run, potassium chloride solution was pipetted from the cup and the oil introduced, also by pipette. To replace the oil, it was first removed by pipette, then 1 to 2 ml. potassium chloride solution was run into the calomel electrode from the top to force out any boundary potassium chloride. This was withdrawn, in turn, from the cup, and a new sample of oil added.

The compound being studied was made up in 0.01, 0.001, and, in some cases, 0.0001 molar solutions, the solvent being a solution of potassium chloride, 0.756 Gm./liter, or sodium benzoate, 0.001 molar. The central beaker of the cell was filled with 100 ml. of this salt solution and stirred until the salt distribution between the oil and the water reached equilibrium, and the potential difference was then measured. Into this was pipetted an amount of drug solution necessary to give the desired concentration, and the potential difference was again observed. The difference between this and the initial readings represented the change in potential difference due to concentration. More of the drug solution was then added until the desired next higher concentration was reached. Since the same salt solution served both as zero reading and as solvent for the compound studied, the salt concentration always remained the same throughout a series of readings. The oils used were guaiacol and nitrobenzene, each freshly distilled.

The data are summarized graphically. Figure 1

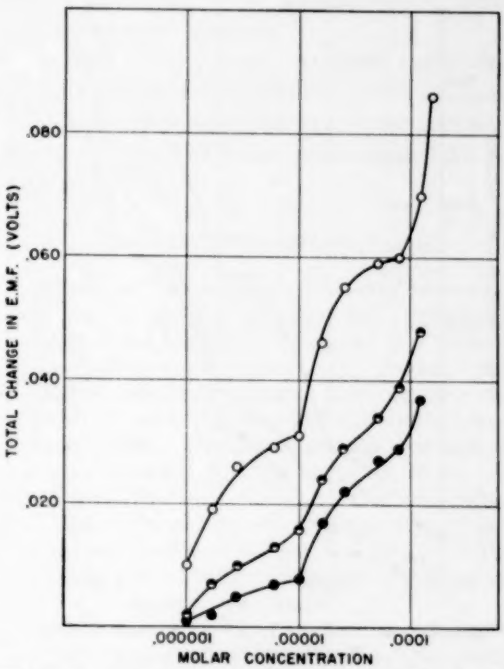

Figure 1.—⊖, propadrine HGl; ●, propadrine base ○, hexadrine HCl.

shows the results obtained with propadrine base, its hydrochloride and hexadrine hydrochloride (C_6H_5—CHOH—CH—C_4H_9); nitrobenzene was

$NH_2 \cdot HCl$

used in the cup and sodium benzoate as the solution. Hexadrine hydrochloride gave, over a wide range of concentrations, a much greater change in voltage than the propadrine hydrochloride. It will be noted that the curves have approximately the same shape and slope. The differences in voltage correspond with the known toxicity of the compounds, the higher homolog being the more toxic (8). Propadrine and its salt produce approximately the same voltage change; this indicates an interchange of ions in the nitrobenzene.

Figure 3 shows the results obtained with propadrine, butadrine, and pentadrine (C_6H_5—CHOH—CH(NH$_2$)—R, R = CH_3, C_2H_5, and n-C_3H_7, respectively) hydrochlorides, using guaiacol and potassium chloride solution. Considered from the point of toxicity, the curves are in proper order, since pentadrine is about three times more toxic than propadrine (8).

Figure 2 shows the results obtained with choline chloride, methylcholine chloride and "triethylcholine" chloride, $HOCH_2CH_2$—$N(C_2H_5)_3Cl$, with sodium benzoate as the solution and nitrobenzene as the oil. Again, the change in voltage gives no indication of physiological activity, but the compound showing the greatest change in voltage is the most toxic.

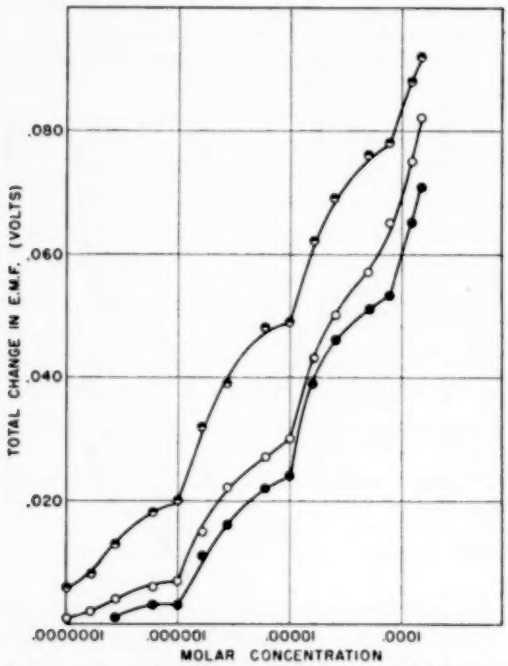

Figure 2.—●, choline Cl; ○, beta methyl choline Cl; ◓, triethyl choline Cl.

In Fig. 3 are shown also the results obtained with cocaine hydrochloride and papaverine hydrochloride, the cell combination being guaiacol and potassium chloride. Here again, the more toxic cocaine produces the greater change in voltage.

DISCUSSION

These results, along with other data not described here, show that it is not yet possible to predict biological activity merely by measuring the change a compound produces in the electromotive force of a "model cell." Possibly the development of a more ideal cell may make for better correlation. Or perhaps the interesting correlations that have thus far been observed, e.g., those shown in Table I, are receiving incomplete or even misleading interpretations. Further studies with homologous series, as employed here, may clarify these points. Our results show only an approximate parallelism between the change in E. M. F. and the relative toxicities of homologous series.

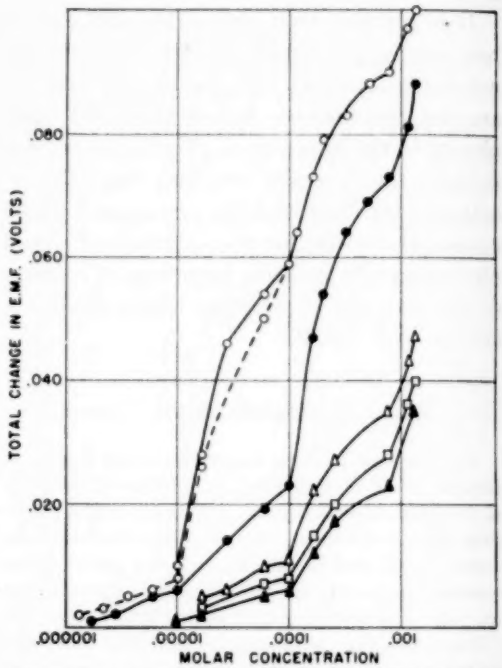

Figure 3.—○, cocaine HCl; ●, papaverine HCl; ▲, propadrine HCl; □, butadrine HCl; △, pentadrine.

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A Study of the Microbiological Assay of Riboflavin*

By HAROLD W. WOODSON,† ERNST R. KIRCH, and OLAF BERGEIM

The values determined using a mixture of twenty amino acids compared favorably with those obtained using the customary media containing alkali-treated peptone or casein hydrolyzate. For the riboflavin assay a new test organism, *Lactobacillus delbrückii*, gave essentially the same growth responses as the standard organism, *Lactobacillus casei*.

KNOWLEDGE of the growth factor requirements of various lactic acid bacteria, especially *Lactobacillus casei* and *Lactobacillus arabinosus 17-5*, has led to the development of useful microbiological methods for the quantitative determination of most of the B vitamins.

Microbiological assays of riboflavin, using as the test organism, *Lactobacillus casei*, have been adopted in many laboratories using the original method of Snell and Strong (1) or the modified medium of Silber and Unna (2). Frequency of irregular standard curves and discordant results are some of the difficulties which have been observed with the Snell and Strong medium, which contains alkali treated-photolyzed peptone and lead-treated yeast supplement. This has led to studies on the improvement of media for this determination, with the object of obtaining greater regularity and sensitivity in the response of the microorganism to riboflavin. In all of the suggested media along with the known growth factors are one or more materials of variable composition, i.e., hydrolyzed casein, photolyzed peptone, and yeast supplement. Recent studies on the amino acid requirements of *Lactobacillus casei* (3) and *Lactobacillus delbrückii LD5* (4), and the use of these organisms in microbiological amino acid assays (5–7) have suggested the possibility of using a completely synthetic medium of known and constant composition for vitamin assays.

Tomlinson and Peterson (8), in their experiments with a synthetic medium for the determination of biotin, suggest that the same medium could be used for the assay of riboflavin, pantothenic acid, and nicotinic acid. However, because of the longer time required (four days) and the possibility of other substances present in natural materials affecting the results, further experiments would be needed before adopting a synthetic medium for such assays.

In the present work, we were interested in the development of an amino acid medium for use in the determination of riboflavin and the application of *Lactobacillus delbrückii* to this assay.

EXPERIMENTAL

Organisms.- The organisms used are *Lactobacillus casei* and *Lactobacillus delbrückii* LD5. Stab stock cultures of the organisms are carried in a medium of the following composition: 1% glucose, 1.5% agar, and 1% yeast extract. Stock cultures are transferred weekly and are stored in the refrigerator.

Basal Media. The following media were used in this investigation: Silber-Unna (2) modification of the original Snell and Strong medium (1), Landy and Dicken (9), and the amino acid medium similar to that described by Baumgarten, *et al.* (10). The composition of the amino acid medium is shown in Table I.

This medium is double strength, 5 ml. being used per tube, diluted to 10 ml. with the solution being assayed plus water. The amino acid medium of Baumgarten, *et al.* (10) was selected since their experiments showed that other proposed amino acid media (3–6) did not give maximal growth response with *L. casei*, whereas maximal growth response was obtained with their medium by the addition of thiamine, which acts as a stimulating factor for *L. casei*.

Assay Procedure. Inoculum tubes are prepared by direct transfer from stock culture to 10 ml. of the diluted basal medium containing 1 μg. of riboflavin. After incubation for twenty-four hours at 37°, the culture is centrifuged and the cells are resuspended in 10 ml. of sterile physiological sodium chloride. One drop of this suspension is used per tube for inoculation.

Assays were carried out in 16 × 150-mm. Pyrex bacteriological culture tubes. To establish the standard curve, duplicate tubes were set up containing the following concentrations of riboflavin: 0.00, 0.05, 0.10, 0.15, 0.2, 0.3, and 0.5 μg. of riboflavin.

* Received April 29, 1946, from the Department of Biological Chemistry, University of Illinois College of Medicine, and the Department of Chemistry, University of Illinois College of Pharmacy, Chicago, Illinois.

This paper is based on a thesis submitted by Harold W. Woodson to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Master of Science.

† Holder of the Julius Rosenwald Fund Fellowship, 1945–1946.

Various amounts of the materials to be assayed, estimated to contain between 0.025 to 0.2 μg. of riboflavin, are pipetted into additional tubes. Volumes up to 5 ml. of the unknown material may be used. Unknown samples were run in duplicate at three levels. Unless otherwise noted the tubes were incubated at 37° for seventy-two hours and the amount of lactic acid produced was determined by titration with 0.1 *N* NaOH, bromothymol blue being used as the indicator.

Effect of Increasing Glucose and Buffer Content of Medium.—Following the suggestion of Stokes and Martin (11) the glucose content of the amino acid medium was increased from 1% to 2%, the sodium acetate from 0.6% to 1.8%. Contrary to their findings, there was no increase in acid production; in fact the curves obtained were very erratic.

Recovery Experiments.—Yeast extract (Difco) and a commercial B-complex elixir were assayed for riboflavin content using *L. casei* and *L. delbrückii* and the amino acid medium. Recoveries of riboflavin added to these materials are quantitative within the experimental error of microbiological methods (Table III).

TABLE I.—AMINO ACID BASAL MEDIUM

dl-Alanine	400 mg.	Glucose	20 Gm.
l (+)-Arginine	400 mg.	Sodium acetate (anhydrous)	12 Gm.
dl-Aspartic acid	400 mg.	Adenine	100 mg.
l (−)-Cystine	400 mg.	Guanine	100 mg.
l (+)-Glutamic acid	400 mg.	Uracil	100 mg.
Glycine	400 mg.	Inorganic salt solution A	10 ml.
l (−)-Histidine	400 mg.	Inorganic salt solution B	10 ml.
l (−)-Hydroxyproline	400 mg.	Thiamine	2 mg.
dl-Isoleucine	400 mg.	Pyridoxine	0.4 mg.
l(−)-Leucine	400 mg.	Calcium pantothenate	0.4 mg.
dl-Lysine	400 mg.	Nicotinic acid	0.8 mg.
dl-Methionine	400 mg.	*p*-Aminobenzoic acid	0.4 mg.
dl-Norleucine	400 mg.	Biotin	10 μg.
dl-Phenylalanine	400 mg.	Folic acid[a]	25 μg.
l (−)-Proline	400 mg.	Adjust to *p*H 6.6–6.8	
dl-Serine	400 mg.	Add distilled H_2O to	1000 ml.
dl-Threonine	400 mg.		
l (−)-Tryptophane	400 mg.		
l (−)-Tyrosine	400 mg.		
dl-Valine	400 mg.		

[a] Equivalent to 25 μg. of material of "potency 3100." Kindly supplied by Dr. R. J. Williams of the University of Texas.

Lactobacillus delbrückii as a Test Organism.—Although *Lactobacillus casei* is the standard organism used in the riboflavin assay, it seemed desirable to investigate the possibility of using another lactic acid bacteria. Stokes and Gunness (4, 7) have described the amino acid requirements of *L. delbrückii LD5*, and have used the organism for the assay of aspartic acid and serine. Their work has also confirmed the fact that riboflavin is essential for *L. delbrückii*. Therefore, the growth response of *L. delbrückii* to riboflavin in comparison with *L. casei* on Silber-Unna medium and the amino acid medium was studied.

Comparison of Basal Media.—Growth response of *L. casei* and *L. delbrückii* on the three basal media, previously described, were studied as a controlled experiment. Assay tubes were set up in duplicate and all operations, solutions, and inocula were the same. The results of this experiment, as shown in Table II, indicate that there is fairly close correlation of growth response in the media tested.

Effect of Time on Acid Production.—Since it was previously reported by Tomlinson and Peterson (8) that a four-day incubation period was necessary for satisfactory growth on their synthetic medium for the assay of biotin, experiments were conducted with *L. casei* on the amino acid medium. With an incubation period of ninety-seven hours, practically the same results were obtained as those following the seventy-two hour incubation period.

TABLE II.—COMPARISON OF ACID PRODUCTION WITH DIFFERENT SOURCES OF NITROGEN. ml. 0.1 *N* ACID PER 10 ml. OF CULTURE MEDIUM

γ per ml. Riboflavin	Silber–Unna	Amino Acid	Landy–Dicken
	Lactobacillus casei		
0.00	1.2	1.55	1.4
0.05	3.9	4.85	4.7
0.1	5.5	6.15	6.2
0.15	6.9	6.8	6.6
0.2	8.4	7.6	8.2
0.3	9.5	8.55	9.0
0.5	10.3	9.1	10.5
	Lactobacillus delbrückii		
0.00	1.1	1.55	1.4
0.05	3.6	4.70	4.8
0.1	5.4	5.9	5.8
0.15	6.9	6.7	6.6
0.2	8.2	7.5	7.6
0.3	9.6	9.2	9.0
0.5	9.9	9.6	10.0

Comparative Riboflavin Assays.—In order to have a basis for evaluating the amino acid medium, a number of materials of widely different composition have been assayed in triplicate with this medium, and also that of the Silber-Unna modification (2) of the original Snell and Strong medium (1). In all cases, the

TABLE III.—RECOVERY OF RIBOFLAVIN

Material	Riboflavin Content, γ per Tube	Riboflavin Added, γ per Tube	Total, γ per Tube	Riboflavin Recovered, γ per Tube	Recovery, %
		Amino Acid Medium			
		Lactobacillus casei			
Yeast extract	0.06	0.2	0.26	0.255	98
B-Complex elixir	0.11	0.1	0.21	0.205	98
		Lactobacillus delbrückii			
Yeast extract	0.07	0.1	0.17	0.17	100
B-Complex elixir	0.11	0.1	0.21	0.19	90

values for riboflavin content were in essential agreement with the results obtained by the Silber-Unna medium (Table IV). The assays of the milk, urine, and yeast extract were carried out on the untreated material diluted to the proper concentration.

TABLE IV.—COMPARATIVE RIBOFLAVIN ASSAYS ON VARIOUS MATERIALS

Material	Silber–Unna Medium, *L. casei*, μg. per Gm. or Ml.	Amino Acid Medium *L. casei*, μg. per Gm. or Ml.
Milk	2.06	2.31
Urine	1.50	1.50
Yeast extract	55.00	60.00

SUMMARY

1. An amino acid medium is described for use in the microbiological assay of riboflavin. The alkali-treated peptone or casein hydrolyzate of the usual media is replaced by a mixture of 20 amino acids at the level of 2 mg. per tube of 10 ml. There is no improvement in the medium by increasing the glucose or buffer capacity.

2. A new test organism for the riboflavin assay, *Lactobacillus delbrückii*, is described which gives comparable growth responses with the standard organism, *Lactobacillus casei*.

3. Various materials, assayed with the amino acid medium, gave values which compared favorably with those obtained by a standard medium.

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Book Reviews

American Pharmacy. Edited by R. A. LYMAN. J. B. Lippincott Co., Philadelphia, 1945. xix + 545 pp. 18 × 26 cm. 197 illus. Price, $8.00.

American Pharmacy is the result of the efforts of twenty-eight men in pharmacy to produce a modern textbook for beginning students in pharmacy. The book is divided into three parts comprising thirty chapters and covering 525 pages of text. In producing this work the following points were considered:

The book was to be (1) primarily a teaching tool and not a book of encyclopedic nature, (2) a reference work for the practicing pharmacist and others in the medical field, (3) modern in every respect, with obsolete material being deleted, and (4) concise and not a reproduction of the official books.

In selecting the various authors an at-

tempt was made to secure men who were specialists in a particular phase of pharmacy and who were cognizant of the needs of pharmacy as a whole.

Since this book is intended primarily as a teaching aid, the comments of the review are so directed.

Part one of the book deals with the Fundamental Principles and Processes of Pharmacy. These are Metrology; Specific Gravity and Specific Volume; Heat and Refrigeration; Purification and Clarification; The Mechanical Subdivision of Drugs; Solutions; Colloids, Emulsions and Suspensions; Extractions; Bacteriologic Technique; and Preservation and Packaging. Of these chapters, Heat and Refrigeration; Solutions; Extractions; and Mechanical Subdivision of Drugs are good for freshman study. Metrology and Specific Gravity and Specific Volume are a little brief. They do not include a sufficient number of illustrations to aid the student in mastering these subjects. The chapters on Purification and Clarification; and Colloids, Emulsions and Suspensions are excellent, but they are probably too advanced for beginning students. There seems to be little or no justification for the chapter on Bacteriologic Technique since this subject is taught in a separate course and later than the freshman year in most colleges.

Part two of the book deals with Pharmaceutical Preparations. The material is grouped under the conventional headings, such as Waters, Syrups and Juices; Solutions; Injections; Infusions and Decoctions; Mucilages, Creams, Glycerogelatins, Glycerites and Collodion; Mixtures, Magmas and Gels; Soaps and Oleates; Liniments, Lotions, Petroxolins, and Sprays; Ointments, Cerates, Plasters, and Cataplasms; Emulsions; Suppositories; Spirits and Elixirs; Vinegars, Tinctures, Fluidextracts, Fluidglycerates and Extracts; Resins and Oleoresins; Masses, Pills, Troches and Tablets; Powders, Capsules, Cachets, Oil Sugars, Candy Medication and Triturations; and Effervescent Salts. In general, each of these subjects is treated well. There are some differences among the authors in the amount of emphasis placed on the techniques involved in the preparation of these pharmaceuticals and some authors seem to stress the use of the preparation more than the manufacture of it. Here again it is noted that there is a wide difference in the levels of discussion of each group of preparations. For example, the chapter on Ointments, Cerates, Plasters and Cataplasms is excellent but hardly within the grasp of a student who is just starting his pharmaceutical studies. The chapter on Soaps and Oleates is of a similar nature. The order of arrangement of the chapters is purely arbitrary and they do not seem to have any particular connection with each other. Not all official items are discussed; this is particularly true of the Chapter on Tinctures, Fluidextracts, etc.

Part three of the book contains chapters on Vitamins; Hormones and Endocrine Glands; and Introduction to Biologicals. These chapters are considered all too brief to be of value in pharmaceutical education, particularly in those colleges that give two or more credit hours in each of these areas. Here again, this material is better suited to advanced students.

However, the book should serve as a valuable reference to students, pharmacists, and others interested in the medical sciences.—E. P. GUTH.

Howell's Textbook of Physiology, 15th Edition. Edited by J. F. FULTON, with ten collaborators. W. B. Saunders Co., Philadelphia, 1946. xxxv + 1304 pp. 15 x 23 cm. Price, $8.00.

When a textbook reaches its fifteenth edition, it would scarcely seem necessary for it to be reviewed. However, *Howell's Textbook of Physiology*, with this edition, has been rewritten completely by ten collaborators under the able editorship of Dr. J. F. Fulton.

The text covers the entire field of physiology, and the sections are developed in the usual pattern. Such an extensive book cannot be reviewed in detail with satisfaction; it is rather to be read and used. In short, the book is thoroughly modernized, and although written by many authors, its continuity is excellent.—MELVIN W. GREEN.